



# **Nidacon** Technical Product Manual

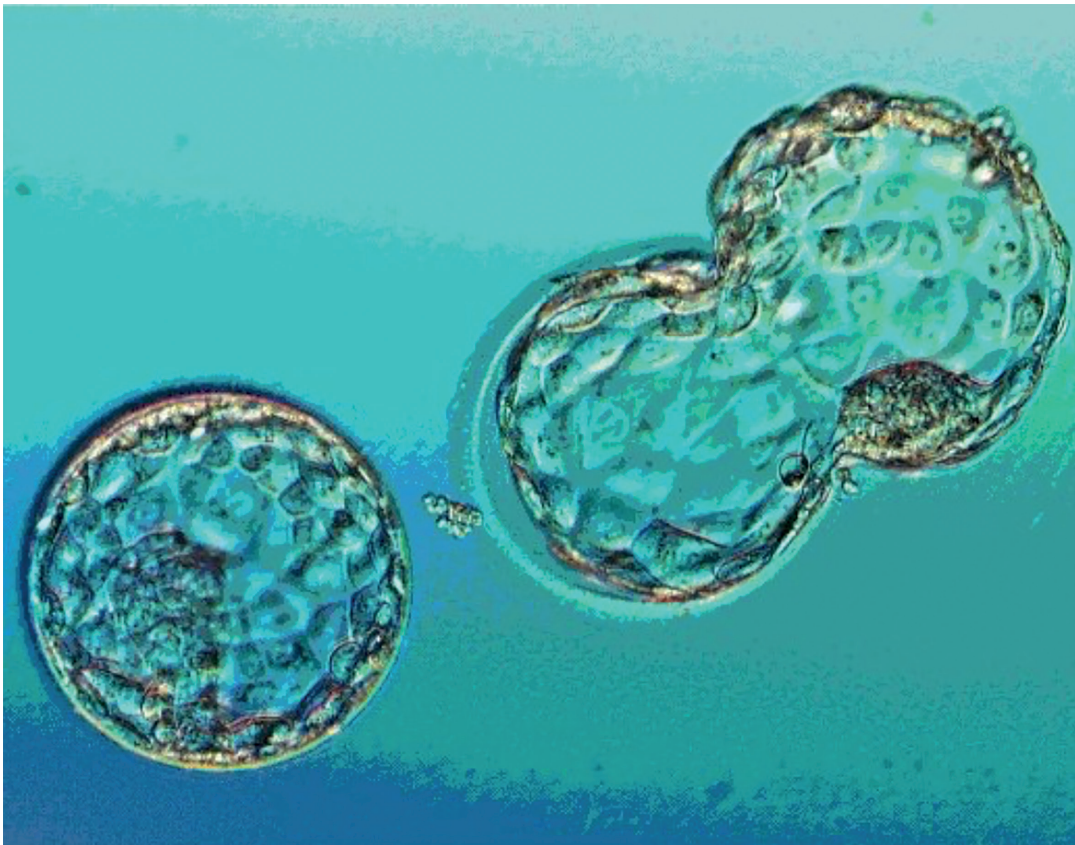


## Nidation

1. The building of a nidus, a nest, as with birds.
2. Implantation of the fertilized ovum (zygote) and the building of a nest in the endometrium, the placenta.

## Conception

1. The union of male and female gametes, the sperm and egg.
2. An impression or idea



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All information regarding products and procedures in the technical product manual corresponds to other information from Nidacon, including inserts at the date of issuing. 2022-04-26

### Introduction

Nidacon International AB manufactures and sells Medical Devices mainly for Assisted Reproduction Technologies (ART), with IVF, ICSI and insemination (IUI) solutions. The company was founded in 1996 by Assoc. Prof. Paul V. Holmes MSc, PhD, DrMedSc, an embryologist and endocrinologist from the Dept. of Obstetrics and Gynaecology at Sahlgrenska University Hospital in Gothenburg, Sweden.

Nidacon considers many different factors when designing its products. We hope that the attention to detail has helped to create products which will lead to better results. We aim to work in close relation with our customers; they are the cornerstones of our research department.

We take pride in the development of our products and make sure we respond to the needs of our customers and research colleagues. All our products are developed in close cooperation with professionals in the different fields.

One of the first products to result from the company's research and development, PureSperm™, was introduced onto the market in November 1996. It has gained rapid acceptance and is now the global market leader for isolation and preparation of sperm used in human assisted reproduction. It was the first product of its kind to achieve both 510(k) clearance from the US FDA and CE marking with the European authorities.

#### Corporate Social Responsibility - CSR

*We shall contribute to a sustainable world by taking responsibility for human welfare and our impact on the environment and society as a whole.*

In ecology, sustainability is the capacity to endure; it is how biological systems remain diverse and productive indefinitely. In more general terms, sustainability is the endurance of systems and pro-

cesses. The principle for sustainability is sustainable development, which includes the four interconnected domains: ecology, economics, politics and culture.

Nidacon believes in the importance of our legacy and works actively to make a positive imprint and to take responsibility for our impact on our world. A more conscious use of resources is essential in order to maintain healthy ecosystems and environments. Having a well-functioning system for waste recycling was therefore a natural step for us.

We investigated our express deliveries and came to the conclusion that we should compensate for carbon emissions. Therefore from now on, we pay an extra fee for every package that is shipped from us. UPS has a program called "UPS Carbon Neutral" which we have joined and, for all other deliveries, we compensate by planting trees through WWF.

Since we are all helping couples to fulfill their dreams of having a family, we feel that we have an extra responsibility for the children in the world. We are therefore supporting an organization called "We care". Today they have three ongoing projects for "street children" in Nepal. We put a lot of effort into choosing which organizations/projects we support but are open to your suggestions. Please, contact us with your thoughts!

We are also proud to be a member of an organization called CSR West Sweden which focuses on global sustainability within all the four interconnected domains mentioned above. We attended a very interesting seminar based on KPMG's study on Global sustainability "Megaforces". To ensure our continual development we will keep attending seminars, courses and conferences with this focus.

### Quality

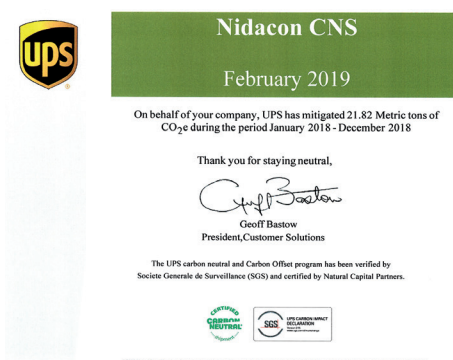
Nidacon is certified according to SS-EN ISO 13485 (implemented 2003-08-15). The management system secures continued development of the organisation.

We register our products according to the valid directives and requirements for all different countries. This also ensures our high quality on the market and it shall continue to be our beacon.

Nidacon intends to always maintain the high quality of its products and, in order to achieve this, all batches are tested at Nidacon before they are cle-

ared for the market. Sterility controls are performed on each batch manufactured, the endotoxin level is measured and biological efficacy tests are carried out. A batch is only released for sale if it meets specific criteria.

Each batch is accompanied by a quality assurance certificate which records the results of the tests. Using this rigorous quality control system, we ensure that each batch meets the correct standards. Consequently the customers are secure in the knowledge that our products are reliable and will provide good results when used correctly.





### Quality Assurance tests

#### Physical analyses

*pH* – tested on every batch during production and after bottled product at room temperature in air.

*Osmolality* – tested on every batch during production and after.

#### Sterility and toxin analyses

*Microbiological growth control* – performed after production of a batch and involve bacterial and fungal growth assays. The assays are performed under a period of 3 weeks in order to be able to detect any growth. They are done by the Bacteriological Laboratory of Sahlgrenska University Hospital, Gothenburg, Sweden.

*Endotoxin detection* – This assay is done with an FDA-approved, Limulus Amoebocyte Lysate (LAL) test using a quantitative spectrophotometric method in order to obtain real values with the units EU/mL, according to the U.S. Pharmacopoeia. The test is done by the Microbiology Institute at the Sahlgrenska University Hospital of Gothenburg, Sweden.

#### Biological analyses

*Human sperm test* – The biological efficacy assay involves assessment, motility and viability, measured both subjectively and using computer assisted sperm analysis (Hamilton-Thorne, IVOS). Each batch is tested biologically using human semen samples. All data are recorded from before and after the separation and purification, and are compared to the control, i.e. using an earlier, already approved batch.

*Human Sperm Survival test for oil* – Prepared sperm are covered by oil and incubated overnight in 37°C, 5-6% CO<sub>2</sub>. Percentage of motile sperm on day 2.

*Mouse Embryo Assay (MEA) for bottles etc.* – is used to assess the in vitro growth and development of pre-implantation embryos exposed to the test item. The assay predicts embryo toxicities in medical devices or related products to be used for assisted reproductive technology (ART).

*Mouse Embryo IVF Assay for media* – a sensitive assay mimicking the human IVF procedure. The preferred assay to screen assisted reproductive technology supplies for toxicities impairing male and female gamete fecundability and subsequent embryo development capacity.

*Peroxide analyses* – Peroxide (e.g. hydrogen peroxide H<sub>2</sub>O<sub>2</sub>) is one of the key reactive

oxygen species formed under oxidative stress conditions and have been shown in several publications to affect embryo culture (ref. 26, 27). We measure all Nidoil batches using a QuantiChrom™ Peroxide Assay. A kit designed to measure peroxide concentration using the chromogenic Fe<sup>3+</sup>-xylenol orange reaction.

*Functional analysis/Efficacy test* – used to prove the efficacy and function of the products.

*Visual control* – constant visual control during production, filling, labeling and final control of chosen ready packages.



### Shelf life

Nidacon is conscious of customer requirements and always tries to provide products which are convenient. This convenience includes ease of transportation and long shelf life. Therefore, most of the products have a shelf life of one to two years from production at 17-27°C.

After opening the products should be stored at 2-8°C when not in use, except for SpermVitalStain™ which is stored at 17-27°C even after opening.

### Packaging

The packaging for Nidacon's products has received the same care and attention to detail as the design of the products.

**Bottles** – For most of our products we have chosen borosilicate glass instead of sodium silicate glass to avoid the leaching of sodium from the bottles into the contents during the long shelf life. Research in our laboratory has shown that sufficient sodium ions can leach from a sodium silicate bottle to have a negative effect on the development of two-cell mouse embryos. Therefore, we avoid exposing all cells to raised sodium-ion levels in the products by packaging in borosilicate glass.

We have also recognized the opportunity to package some of our products in bottles made from the plastic resin PET. The glass-like clarity, toughness, and excellent gas-barrier properties of PET makes it an outstanding choice for Nidacon media.

The same shelf life applies even after opening if the products are handled under aseptic conditions.

All ingredients are chosen for their temperature tolerance and their stability in aqueous solution. Rigorous shelf life testing has been carried out in Nidacon's laboratory to ensure that the theoretical stability of the salt formulations is matched by their actual stability when combined in the product.

The bottles we use are made only from virgin PET resin and provide a lightweight, shatterproof alternative to glass and made for easy recycling. PET is non-cytotoxic and has been shown through MEA testing to be biologically equivalent to Type 1 borosilicate glass bottles.

**Stoppers** – Based on embryo-toxicity testing of three types of commercially available rubber stoppers approved for pharmaceutical use today, Nidacon chose silicone rubber as the material for the stoppers. We found that both natural latex rubber and butyl rubber are toxic to embryos, preventing development and possibly causing embryonic death. Silicone rubber did not have any detrimental effect, allowing embryonic development and hatching to proceed as usual. Therefore, stoppers made from pharmaceutical silicone rubber were chosen for our products.





### Background

Under normal physiological circumstances, sperm undergo a series of maturation changes after ejaculation which enables them to negotiate the different sections of the female reproductive tract, and eventually locate and fertilise the egg. If sperm are to be used for ART, it is essential that any product which is used for sperm preparation must match the sperm's physiological requirements as closely as possible. If sperm are stimulated excessively, particularly ionically, they become "hyperactive", a process which results in the sperm using up its energy resources and dying before fertilisation is achieved.

Therefore, the pH and osmolality of the sperm solutions must be adjusted very specifically to avoid ionic shock and subsequent hyperactivation.

### Product composition

The component salts of Nidacon's products are balanced with specific regard to the ion composition of both the ejaculate and the female reproductive tract. This balance ensures a smooth transition from ejaculate to fertilisation medium via the gradient and wash.

### Buffer

Fluctuations in pH and temperature are detrimental to sperm survival on the bench. In addition, HEPES has an anti-oxidant effect, reducing reactive oxygen species (ROS) which can be damaging in the sperm preparation.

The zwitterion buffer, HEPES, is included to maintain the pH of the PureSperm™ gradient and PureSperm™ Wash while working with the sperm on the bench. Fluids designed to maintain pH in a CO<sub>2</sub> environment, i.e. in the incubator, are unsuitable for use outside the incubator as they do not possess sufficient buffering capacity to maintain the pH.

### Glucose

Glucose is the primary energy substrate available to sperm in the human female reproductive tract and is therefore a component of PureSperm™ products.

### Antibiotics

Antibiotics are not included in our products for several reasons. Penicillin G, a commonly used antibiotic in cell culture medium, only lasts for approximately 10 days in aqueous solution, being inactivated after this time and the degradation products are cell-toxic. Furthermore, this antibiotic is ineffective against some of the bacteria most commonly

found in semen. Streptomycin and gentamycin are cytotoxic. Gentamicin, in particular, has been shown to be toxic to embryos.

Therefore, it seems prudent to avoid including potentially spermicidal components in sperm preparation fluids. Moreover, bacterial contamination in the ejaculate is removed by density gradient preparation. Therefore, the absence of antibiotics in the gradient will not be detrimental to the sperm preparation, and avoids exposing the sperm to potentially toxic compounds. (Ref. 1-5)

### Additives and Phenol Red

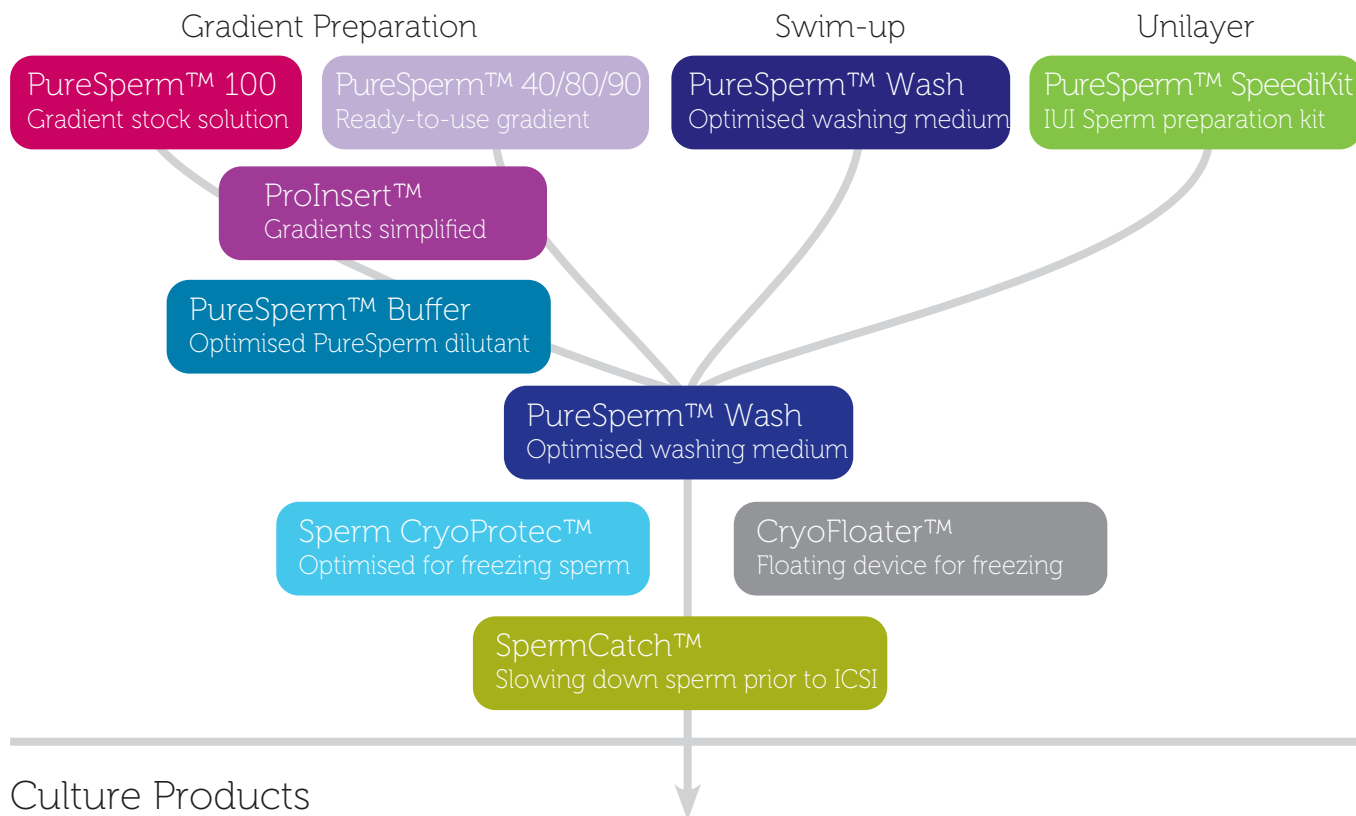
No preservatives or unstable ingredients are added to Nidacon products. In addition, we have decided not to use phenol red in our media, since it has been proven to have estrogenic effects. Gametes have receptors for estrogen and they can be affected by its presence. For instance, it has been shown that estrogen inhibits sperm motility and the acrosome reaction. (Ref. 14)



## Diagnostic Products

Sperm VitalStain™  
One-step vitality stain

## Sperm Preparation



## Culture Products

VitriBlast™  
Vitrification of blastocysts

ThermoBlast™  
Warming of vitrified blastocysts

NidOil™  
Overlay during embryo culture

## Ordering information

Cat. No.	Description	Size
PSK-020	PureSperm™ 40/80	2 x 20 mL
PS40-100	PureSperm™ 40	100 mL
PS80-100	PureSperm™ 80	100 mL
PS90-100	PureSperm™ 90	100 mL
PS100-100	PureSperm™ 100	100 mL
PS100-250	PureSperm™ 100	250 mL
PS100-1000K	PureSperm™ 100	4 x 250 mL
PSB-100	PureSperm™ Buffer	100 mL
PSW-100	PureSperm™ Wash	100 mL
PSW-020	PureSperm™ Wash	2 x 20 mL
PSSK-010	PureSperm™ Speedikit	10 patient preps

Cat. No.	Description	Size
SC-100	SpermCatch™	6 x 100 µL
SCP-020	Sperm CryoProtec™	2 x 20 mL
NO-100	NidOil™	100 mL
NO-400K	NidOil™	4 x 100 mL
SVS-010	Sperm VitalStain™	2 x 10 mL
VBK-010	VitriBlast™ Kit	3 x 10 mL
TBK-010	ThermoBlast™ Kit	4 x 10 mL
PI15-5	ProInsert™	5 kits
CFV-001	CryoFloater™	vial 1 pcs
CFV-001	CryoFloater™	straw 1 pcs





## PureSperm™ 100

Optimized for the preparation of discontinuous density gradients for the separation and purification of human sperm. Sterile silane-coated, silica colloid in an isotonic salt solution.

Shelf life 2 years.

*QA Sterility – Osmolality – Endotoxin  
pH – Human Sperm Survival*

### Components

NaCl	EDTA
KCl	CaCl
HEPES	Silane-Coated Silica
Glucose	Purified water



## PureSperm™ 40

## PureSperm™ 80

## PureSperm™ 90

Ready-to-use density gradient solutions, 40, 80 and 90%. They make work in the lab easier and minimizes the risk for mistakes.

Shelf life 2 years.

*QA Sterility – Osmolality – Endotoxin  
pH – Human Sperm Survival*

### Components

NaCl	Lactate
KCl	Pyruvate
HEPES	Silane-Coated Silica
Glucose	CaCl
EDTA	Purified water



## PureSperm™ Buffer

Balanced salt solution designed specifically for diluting PureSperm™ 100 to make up the different discontinuous gradient layers. The optimised formulation of PureSperm™ Buffer is designed to maximise sperm survival during gradient centrifugation.

Shelf life 2 years.

*QA Sterility – Osmolality – Endotoxin  
pH – Human Sperm Survival*

### Components

NaCl	Lactate
KCl	Pyruvate
HEPES	Citrate
Glucose	Purified water
EDTA	

## Products



### ProInsert™

The ProInsert™ reduces the risk of recontamination of the sperm pellet during sperm retrieval after density gradient separation. The ProInsert™ is safe and easy-to-use, designed for use with Nidacon products.

Shelf life 2 years.

**QA** *Efficacy test – Mouse Embryo Assay  
Sterility – Human Sperm Survival*

#### Components

Centrifuge tube containing the ProInsert™  
Pellet Retrieval Pipette



### PureSperm™ Wash

Sterile isotonic salt solution. Optimized for washing the sperm recovered from density gradient preparations, for use in swim-up procedures, for extension of sperm prior to IUI or as a medium for maintaining sperm.

Shelf life 1 years.

**QA** *Sterility – Osmolality – Endotoxin  
pH – Human Sperm Survival*

#### Components

NaCl	Glucose
KCl	EDTA
MgSO <sub>4</sub>	Lactate
KH <sub>2</sub> PO <sub>4</sub>	hSA
NaHCO <sub>3</sub>	Pyruvate
HEPES	Purified water

### PureSperm™ SpeediKit

A kit that provides the components required to prepare 10 sperm samples for IUI. It contains ready-to-use tubes for a single layer centrifugation and ready-to-use tubes with PureSperm™ Wash for the washing of the pellet after centrifugation. A perfect product for the small clinic, 10 patients/kit.

Shelf life 1 years.

**QA** *Sterility – Osmolality – Endotoxin  
pH – HumanSperm Survival*

#### Components

NaCl	EDTA
KCl	Lactate
MgSO <sub>4</sub>	Pyruvate
NaHCO <sub>3</sub>	Silane-Coated Silica
HEPES	hSA
Glucose	Purified water



## Sperm CryoProtect™

Sterile salt solution containing glycerol, optimised for freezing both gradient-prepared sperm and unprocessed ejaculates. Nidacon recommends the nitrogen-vapour freezing technique, since it provides the best results after thawing.

Shelf life 1 years.

**QA** Sterility – Endotoxin – pH  
Recovery rate after freezing and thawing

### Components

NaCl	Glucose
KCl	EDTA
MgSO <sub>4</sub>	Lactate
KH <sub>2</sub> PO <sub>4</sub>	Pyruvate
NaHCO <sub>3</sub>	Glycerol
HEPES	Purified water



## CryoFloater™

A floating device, used when cryo preserving semen or prepared sperm in liquid nitrogen. It provides a constant distance between the sample and the nitrogen surface, to standardize the freezing rate.

Available both for vials and straws.

**QA** – Visual inspection

### Components

Polyethylene foam



## Sperm VitalStain™

One step staining technique for assessment of sperm vitality, a basic tool for semen analysis.

Shelf life 1 years.

**QA** pH – Functional analysis

### Components

NaCl	Formalin
Eosin	Purified water
Nigrosine	



## SpermCatch™

For slowing down sperm prior to ICSI without using polyvinylpyrrolidone (PVP). To avoid ICSI injection of PVP, it contains only natural products for increasing the viscosity.

Shelf life 1 years.

**QA** Sterility – Osmolality – Endotoxin  
pH – Human Sperm Survival

### Components

NaCl	Pyruvate
MgSO <sub>4</sub>	Lactate
KCl	EDTA
KH <sub>2</sub> PO <sub>4</sub>	Purified water
Glucose	HEPES
NaHCO <sub>3</sub>	Hyaluronic acid
hSA	



## NidOil™

Sterile, light paraffin oil for use as an overlay during gamete, zygote and pre-embryo culture in the incubator, or during manipulations outside the incubator. No additives, UV-protective packaging.

Shelf life 2 years.

**QA** *Density – Sterility – Endotoxin*  
*– Human Sperm Survival*  
*– Mouse Embryo Assay*  
*– Peroxides Analyses*

### Components

Light paraffin oil



## VitriBlast™

Kit for vitrification of blastocysts based on well tested formulations using DMSO and ethyleneglycol. Numerous publications demonstrate the effectiveness regarding both survival and pregnancy rates.

Shelf life 12 months.

**QA** *Sterility – Osmolality – Endotoxin*  
*pH – Mouse Embryo Assay*

### Components

NaCl	Lactate
KCl	Pyruvate
MgSO <sub>4</sub>	Sucrose
KH <sub>2</sub> PO <sub>4</sub>	Ficoll
NaHCO <sub>3</sub>	DMSO
HEPES	Ethyleneglycol
Glucose	hSA
EDTA	Purified water



## ThermoBlast™

Kit optimised for warming blastocysts vitrified with VitriBlast™ Kit. Ready-to-use solutions.

Shelf life 12 months.

**QA** *Sterility – Osmolality – Endotoxin*  
*pH – Mouse Embryo Assay*

### Components

NaCl	EDTA
KCl	Lactate
MgSO <sub>4</sub>	Pyruvate
KH <sub>2</sub> PO <sub>4</sub>	Sucrose
NaHCO <sub>3</sub>	hSA
HEPES	Purified water
Glucose	



## Background

A normal semen sample (ejaculate) is made up of seminal fluid which contains a number of different cells, cell debris, microbiological and biological substances.

The different cell types contained in semen are normal motile sperm, juvenile sperm and senescent sperm (no fertilisation function) and sperm with DNA breaks. Epithelial cells from the male reproductive tract, male immune cells and cell debris (detritus) are also present in the semen, as are bacteria and possibly viruses.

Moreover, the seminal fluid contains biologicals such as sperm decapacitating factors and reactive

oxygen species (ROS), both of which negatively affect fertilisation.

After ejaculation in vivo, normal sperm quickly migrate from the liquefied semen into the uterine cervix of the female, thereby separating themselves from adverse affects of the factors previously mentioned.

In the andrology laboratory of an IVF-clinic, separation of the normal motile sperm from seminal fluid and its contents can be achieved by using either a "discontinuous density gradient" or a "swim-up". (Ref. 6-7)

### Positive features of a discontinuous density gradient.

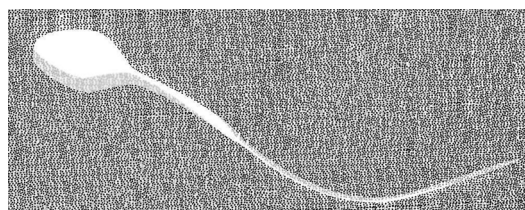
Feature	Density Gradients	Swim-up
Separates motile sperm from other cell types	✓	✓
Separates out immature, aged and dying sperm	✓	—
Separates out morphologically abnormal sperm	✓	—
Separates out sperm with damaged chromatin	✓	—
Removes bacteria, viruses and protozoa	✓	—

If the density gradient has been prepared correctly, the sperm pellet should contain only functional, fertile sperm. (Ref. 19, 20)

## What is PureSperm and how does it work?

PureSperm™ contains colloidal silica which has gone through the process of silanization. A colloid is a dispersion of particles that are small enough to not be affected by the force of gravity ( $\leq 1 \mu\text{m}$ ) but large enough ( $> 1 \text{ nm}$ ) to not be defined as a true solution (The colloidal chemistry of silica). It is this which gives PureSperm™ its specific functional characteristic.

Silica occurs naturally in crystalline form and it is the most abundant component of the earth's crust. It is also known to play an important role in many biological systems and in nature.



However, the silica present in PureSperm™ is manufactured through an industrial process which ensures it meets our requirements and specifications. This synthetic colloidal silica contains amorphous silicon dioxide ( $\text{SiO}_2$ ). The spherical silica particles have a smooth surface and are dispersed in a fluid which is referred to as a silica sol. The fluid in this case is water, and therefore it is better known as an aquasol or a hydrosol. Furthermore, it is a stable dispersion. This means that the solid particles do not settle or agglomerate at any significant rate. The silanization occurs during a special chemical reaction that results in a surface coating of the silica

particles. The silica particles have a negative surface charge to begin with and the silane reduces the charge slightly and induces a steric stabilization of the particles. This means that they are more protected against settling out of the dispersion.

In comparison to the silanized silica, the size of the sperm cell is as follows: the sperm head 3-5  $\mu\text{m}$  in length, 2-3  $\mu\text{m}$  in width, and the tail is 45-50  $\mu\text{m}$  long (PGBLA) see picture below. This is a ratio of approximately 1000:1.

The colloidal solution is then adjusted with different salts in order to have the right physiological composition and osmolality for human spermatozoa, pH and osmotic values are set to lie intermediately between those in the semen (liquified ejaculate, pH 7.8, ~340 mOsmol), from where the sperm cells originate, and those in the fertilisation medium (pH 7.35, 285 mOsmol), where the sperm are placed after separation and purification.

In the formulation for PureSperm products, the three salts ( $\text{NaCl}$ ,  $\text{KCl}$  and  $\text{CaCl}_2$ ) have been added in order to provide the correct levels of sodium, potassium, calcium and chloride ions on the outside of the sperm cell (extracellular environment).

# Density Gradient Preparation

PureSperm™100 PureSperm™40 PureSperm™80 PureSperm™90  
PureSperm™Buffer PureSperm™Wash

## Recommendations

If you have a sample with a high volume (>3mL), you can prepare two PureSperm™ gradients for each semen sample. This reduces the risk of overloading a single gradient, provides security when handling tubes or recovering sperm pellets and provides two tubes to balance the centrifuge rotor.

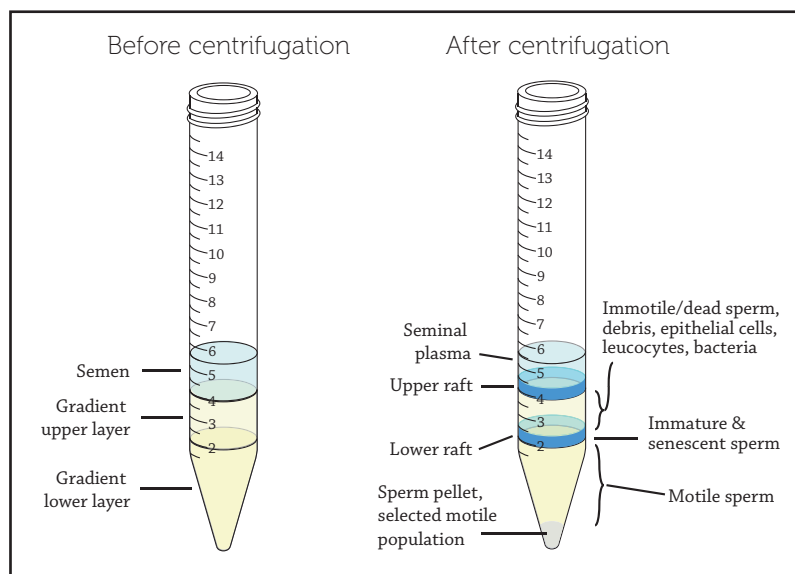
The difference in the results between a 40/80 and a 40/90 gradient is that the higher concentration will give a slightly lower yield but a higher motility. The overall difference is not very large but will give a more specific separation depending on the treatment chosen for the patient.

It is important to have the correct g-force and we advise you to use the equation to make sure it's the recommended g-force for the centrifugation.

To achieve the correct g force:

$$\text{Rpm} = \sqrt{\left[ \frac{g}{(1.118 \times r)} \right]} \times 10^3$$
  
r = rotational radius, the distance (mm) from the centre of the rotor to the bottom of a centrifuge tube in the bucket when raised to horizontal position.

You can also find a calculator on our website [www.nidacon.com/rpm](http://www.nidacon.com/rpm). Put in your numbers and you will receive the desired g-force or RPM.



## General care and use

- All solutions should be brought 17-27°C before use to avoid the temperature fluctuations which are detrimental to sperm survival.
- Open and reseal bottles in a laminar air-flow bench using sterile techniques to avoid contamination.
- Store all opened bottles at 2-8°C after re-sealing.
- The same shelf life applies even after opening for all Nidacon products.

## Tips

- Gradients should be layered immediately prior to use but the different density solutions of PureSperm™ can be prepared in advance, provided that they are stored at 4°C and brought up to room temperature before use.
- Viscous samples can be treated with PureSperm™Buffer. You simply add PureSperm™Buffer to the ejaculate, 1 part PureSperm™Buffer and 3 parts sample, incubate for 15-30 minutes at 37°C and the sample is ready for use.
- When retrieving the pellet after the gradient centrifugation, care must be taken to avoid contaminating the pellet with components of the ejaculate or upper gradient layer. Therefore we recommend that you use a new pipette after removing most of the gradient to avoid contamination, for example, by bacteria.
- To avoid recontamination you can also use ProInsert™ (see procedure p. 17).

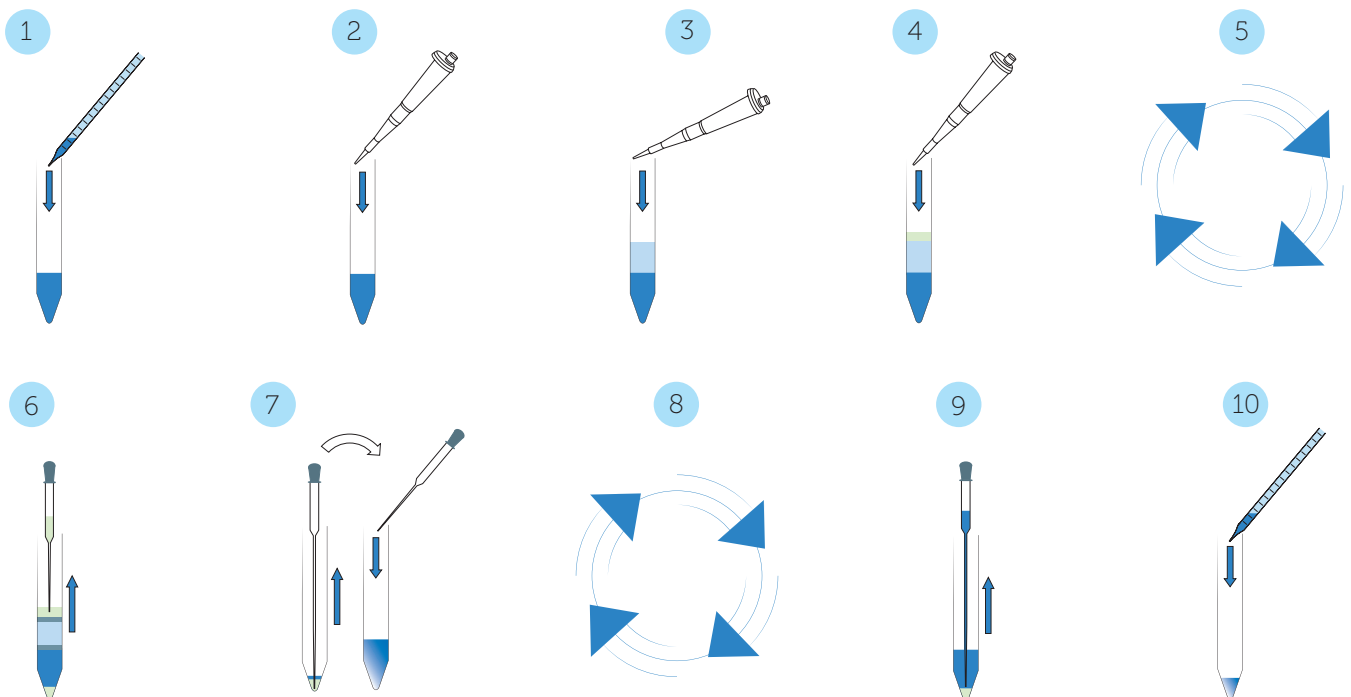
## Reagents and Equipment

PureSperm™ 100 plus PureSperm™ Buffer or  
PureSperm™ 40, 80 and 90  
Sterile Pasteur pipettes

PureSperm™ Wash  
Sterile 2 mL and 10 mL pipettes  
Bench top centrifuge with swing out rotor

## Procedure

1. If you use PureSperm™ 100, dilute with PureSperm™ Buffer to make your gradient solutions, for example add 2 mL PureSperm™ Buffer to 8 mL PureSperm™ 100 to obtain 10 mL 80% PureSperm™. Add 6 mL PureSperm™ Buffer to 4 mL PureSperm™ 100 to obtain 10 mL 40% PureSperm™. Instead you can use the ready-to-use PureSperm™ 40, 80 and 90 solutions.
2. Use a sterile pipette to add 2 mL of the lower layer PureSperm™ (eg 80%) to a conical tube.
3. Use a new pipette to carefully layer 2 mL of the upper layer of PureSperm™ (eg 40%) on top of the lower layer. It is important not to disrupt the two layers and to maintain a sharp interface.
4. Layer the liquefied semen onto the gradient. We recommend that you don't take more than 1.5 mL /gradient or you risk overloading the gradient and not getting a good result.
5. Centrifuge at 300 x g for 20 minutes. Make sure that your centrifuge uses the correct g-force (use equation, p. 14). Do not use the brake.
6. Aspirate in a circular movement from the surface everything except the pellet and 4-6 mm of the lower PureSperm™ layer. If no pellet is seen after centrifugation, remove all fluid except the lowest 0.5 mL.
7. Use a new pipette to aspirate the pellet (or the lowest 0.5 mL). Transfer sperm pellet to a new tube and resuspend pellet in 5 mL PureSperm™ Wash. Always use a new tube with PureSperm™ Wash to avoid contamination from the ejaculate. Combine sperm pellets if double procedure has been used.
8. Centrifuge at 500 x g for 10 minutes. Do not use the brake.
9. Aspirate PureSperm™ Wash supernatant leaving as little liquid as possible above the pellet. If no pellet is seen, leave the bottom 0.25 mL fluid.
10. Resuspend the sperm pellet in a suitable volume of media. The sample is now ready for use.



# IUI preparation

## PureSperm™ SpeediKit

### Background

We especially recommend PureSperm™ SpeediKit for the smaller clinics or for IUI clinics. PureSperm™ SpeediKit is a rapid and efficient alternative to sperm-preps using density 'gradient' centrifugation. Everything is included in a convenient kit form for quick sperm preparation, all based on the effective centrifugation through a single

layer of PureSperm™ colloid, followed by rinsing the sperm with PureSperm™ Wash. The kit contains both the PureSperm™ colloid and the PureSperm™ Wash for 10 patients, already dispensed in centrifuge tubes. You do not need an incubator.

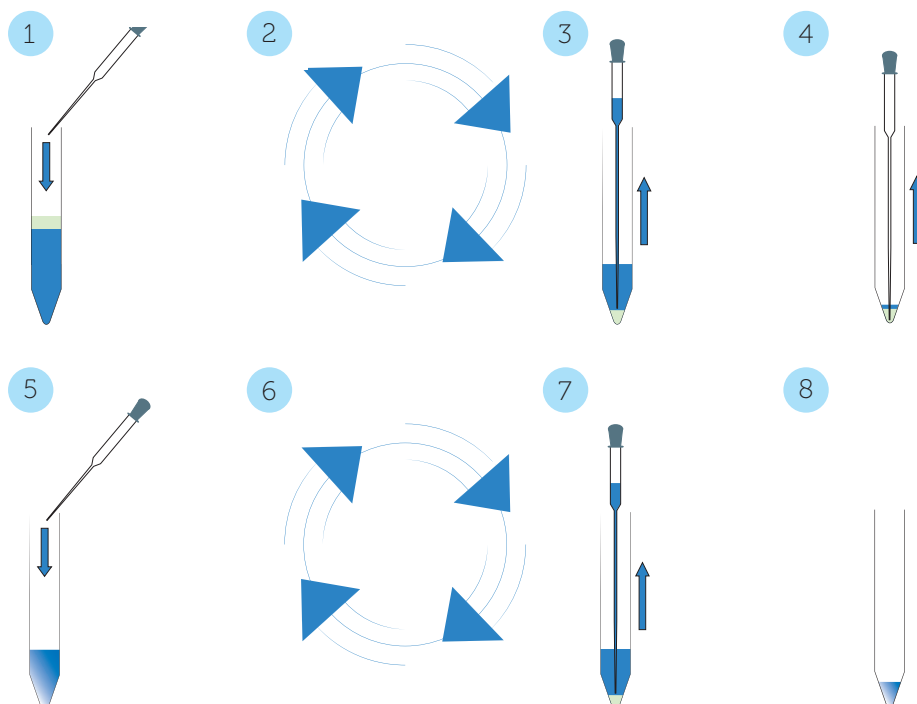
### Reagents and Equipment

Ready-to-use tubes of PureSperm™ Unilayer and PureSperm™ Wash (included in the kit)

Bench top centrifuge with swing-out rotor  
Sterile Pasteur pipettes

### Procedure

1. Use a sterile pipette to carefully layer liquified semen (up to 1.5 mL) on top of the PureSperm™ Unilayer. If you have a sample volume greater than 1.5 mL, use two tubes.
2. Centrifuge at 300 x g for 30 minutes. Do not use the brake.
3. Use a new sterile pipette to aspirate the supernatant, leaving about 5 mm of liquid above the pellet. If no pellet is seen after centrifugation, remove all fluid except the lowest 0.5 mL.
4. Use a new pipette to aspirate the pellet (or the lowest 0.5 mL).
5. Transfer sperm pellet to the tube containing PureSperm™ Wash. Resuspend the sperm.
6. Centrifuge at 500 x g for 10 minutes. Do not use the brake.
7. Use a new pipette to aspirate the supernatant. If no pellet is seen, leave the bottom 0.25 mL fluid.
8. Resuspend the pellet in the remaining PureSperm™ Wash. The sperm preparation is now ready for use.





## ProInsert™

## Background

A density gradient will effectively remove lymphocytes, epithelial cells, abnormal, senescent and immature sperm, cell debris, seminal fluid, bacteria and to some extent viruses. When performing a density gradient the only means of retrieving the sperm pellet at the bottom of the tube is to either, go directly through the layers, disrupt them and risk re-contaminating the pellet, or to remove as much as possible of the layers, go through the remaining

gradient, still risking re-contaminating. Since the gradient will include many risky contaminants, it is crucial that the sperm pellet is not re-contaminated. The ProInsert™ will reduce this re-contamination risk. The insert is included in the centrifuge tube from the start and, after the preparation the pellet can be retrieved through a channel which leads all the way down to the pellet without coming in contact with the now contaminated gradient. (Ref. 21)

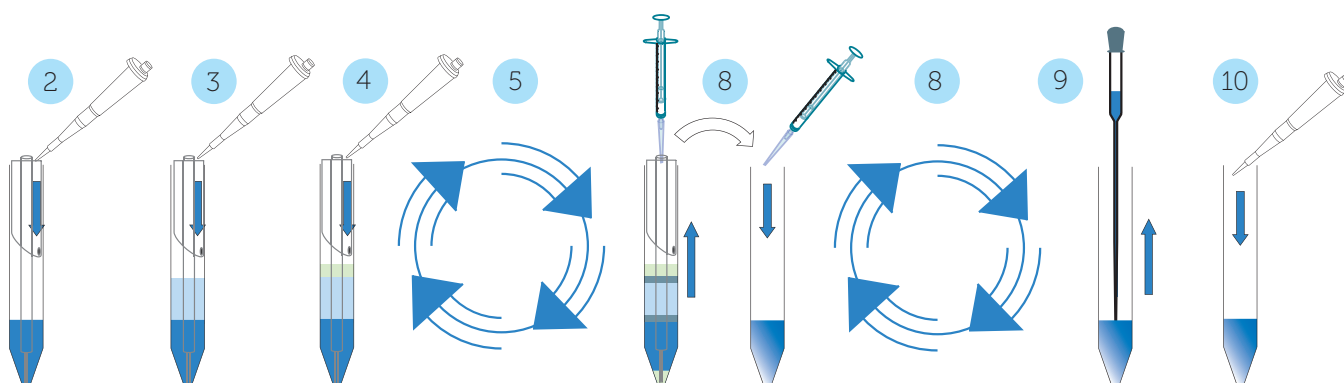
## Reagents and Equipment

Centrifuge tube containing the ProInsert™  
Pellet Retrieval Pipette

Bench top centrifuge with swing-out rotor  
Sterile pipettes

## Procedure for using ProInsert™

1. Open a ProInsert™ Kit and remove the centrifuge tube containing the ProInsert™.
2. Use a sterile pipette to add 2 mL of PureSperm™80 to the outer channel. The gradient material will run down into the ProInsert™ chamber, out through a hole at the bottom of the chamber, and down the wall of the centrifuge tube to form a layer at the bottom of the centrifuge tube.
3. Repeat number 2, using a new sterile pipette tip and PureSperm™40, again via the outer channel.
4. Use a new sterile pipette tip to carefully layer liquefied semen (up to 1.5 mL) again via the outer channel. Take care not to touch the edges of the central channel with the semen.
5. Cap the tube and centrifuge at 300 x g for 20 minutes. Do not use the centrifuge brake. Calculate the correct RPM for your centrifuge (use equation p. 15).
6. Add 5mL PureSperm™Wash to a centrifuge tube (not illustrated).
7. Attach the pellet retrieval pipette from the ProInsert™ Kit to a 1-2 mL syringe (not illustrated).
8. Pass the pipette slowly into the ProInsert™ via the central channel, down to the sperm pellet (see graphic), be careful not to disrupt the pellet. Aspirate the sperm pellet. Retract the pipette until the tip of the pipette is safely above the liquid surface, aspirate a little air and retract the pipette from the central channel. (This procedure is to ensure that no contents in the pipette will be lost during transfer to the PureSperm™ Wash). Transfer the pellet to the tube containing PureSperm™ Wash. Centrifuge at 500 x g for 10 minutes. Do not use the brake.
9. Aspirate the PureSperm™ Wash supernatant, leaving as little liquid as possible above the pellet. If no pellet is seen, leave the bottom 0.25mL fluid.
10. Re-suspend the sperm pellet in a suitable volume of culture/transfer medium (e.g. PureSperm™ Wash) to obtain the required sperm concentration for IVF, ICSI or IUI. The sperm sample is now ready for analysis or use.



# Swim-up

## PureSperm™ Wash

### Background

For most situations Nidacon recommends using a discontinuous density gradient for preparing human sperm from semen. However, many customers at some time need to use the swim-up technique and the most ideal product for this purpose

is PureSperm™ Wash.

PureSperm™ Wash is a salt solution balanced and adjusted for the nutrition and long survival of human sperm. It functions exceedingly well for the swim-up technique.

### Recommendations

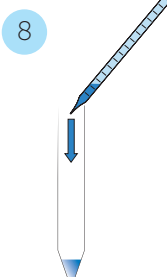
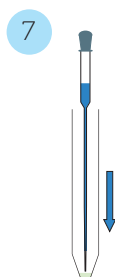
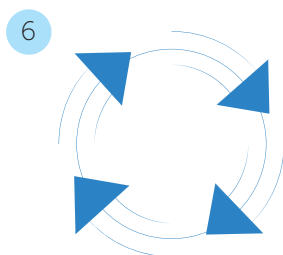
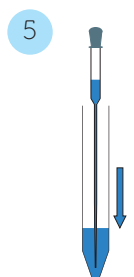
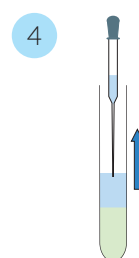
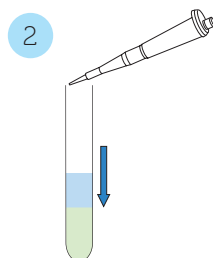
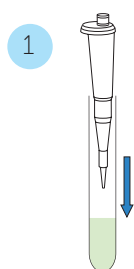
### Reagents and Equipment

PureSperm™ Wash  
Round bottomed centrifuge tubes  
Disposable sterile conical centrifuge tubes  
Sterile pipettes

CO<sub>2</sub> incubator  
Bench top centrifuge with swing out rotor

### Procedure

1. Transfer 1 mL of liquefied semen to a sterile round bottomed centrifuge tube. If the sample is too viscous, try diluting it with PureSperm™ Buffer before.
2. Use a new pipette to carefully layer 1,5 mL PureSperm™ Wash over the semen.
3. Without disturbing the layers, place the centrifuge tube at a 45° angle into a CO<sub>2</sub> incubator, at 37°C for 60 minutes.
4. Carefully remove the uppermost (0,5-1,0 mL) of medium containing motile sperm using a sterile pipette.
5. Place this fluid in a sterile conical centrifuge tube containing 5 mL PureSperm™ Wash.
6. Centrifuge at 500 x g for 10 minutes. Do not use the brake.
7. Aspirate the supernatant, leaving no more than 2 mm depth of liquid above pellet.
8. Resuspend the sperm pellet in a suitable volume of medium to obtain the required sperm concentration. The sample is now ready for analysis or use.



### Tips

- If you have a viscous sample, be extra careful when you remove the upper layer after incubation. It is easy to get hold of the semen sample and disrupt the layers.

## Sperm CryoProtec™

## Background

The cryoprotectant in SpermCryoProtec™ is glycerol, the proportion being reduced as far as possible to minimize toxicity to sperm, while still providing cryoprotection. Moreover, a high

concentration of glucose is present as an osmotic agent to reduce intracellular water; thus diminishing damage due to ice-crystal formation.

## Recommendations

Although it is possible to freeze unprocessed semen, we recommend that you prepare the ejaculate using a PureSperm™ density gradient. This method removes

seminal plasma as well as ROS and their sources, thereby ensuring optimal recovery of motile sperm on thawing.

## Reagents and Equipment

Sperm CryoProtec™ and PureSperm™ Wash  
Sterile pipettes  
Disposable sterile centrifuge tubes (e.g. Falcon 2075)

Disposable sterile cryopreservation vials or plastic straws  
Scissors  
CryoFloater™ (Nidacon)

## Dilution table

Sperm Sample (μL)	SCP™ (μL)	Sperm Sample (μL)	SCP™ (μL)	Sperm Sample (μL)	SCP™ (μL)
100	33	1100	367	2100	700
200	67	1200	400	2200	733
300	100	1300	433	2300	767
400	133	1400	467	2400	800
500	167	1500	500	2500	833
600	200	1600	533	2600	867
700	233	1700	567	2700	900
800	267	1800	600	2800	933
900	300	1900	633	2900	967
1000	333	2000	667	3000	1000

For other volumes than those listed; calculate:  $\text{Volume Sperm Sample} / 3 = \text{Volume SCP}$   
Example:  $300 \mu\text{L Sperm Sample} / 3 = 100 \mu\text{L SCP}$

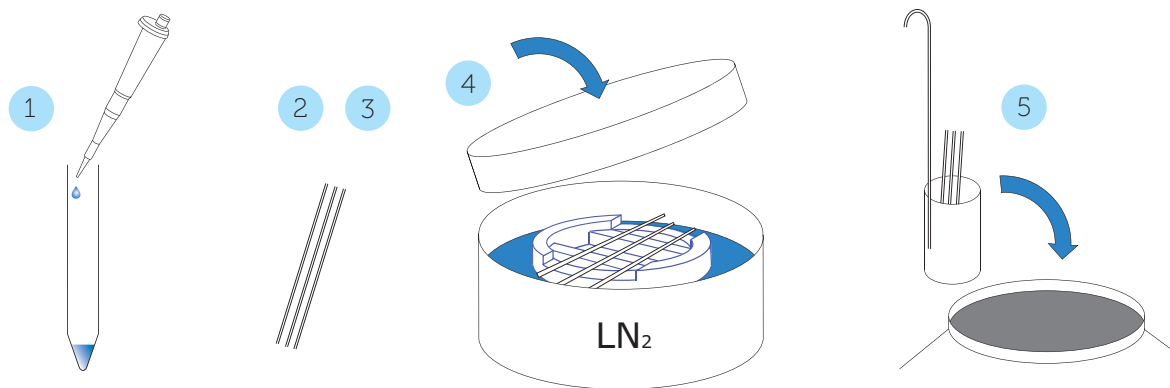
## Tips

- To avoid osmotic shock for the sperm, it is important to slowly mix Sperm CryoProtec™ with your sperm sample.

# Freezing of spermatozoa

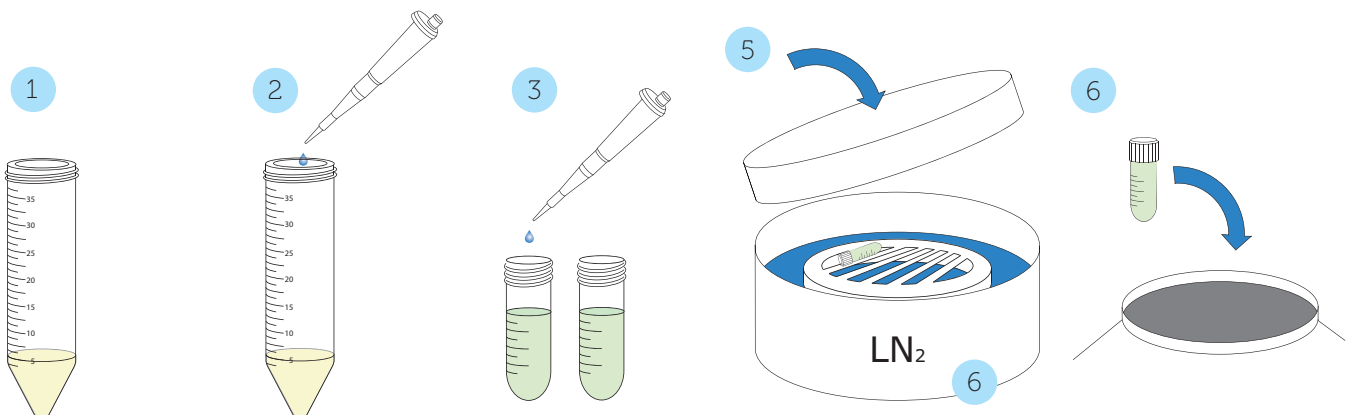
## Processed ejaculate

1. Add 1 part of Sperm CryoProtect™ to 3 parts of sample (see dilution table) ensuring thorough mixing after adding each drop. Mix slowly to avoid osmotic shock.
2. Fill straws with sperm suspension or aliquot into vials. Seal the straws.
3. Equilibrate straws or vials in refrigerator for 10-60 minutes.
4. Place the straws horizontally in nitrogen vapour, above the liquid nitrogen surface on a piece of styrofoam (CryoFloater™). Put on a lid and leave for 10-30 minutes.
5. Transfer the straws quickly into the liquid nitrogen and, thereafter, store in liquid nitrogen. Do not touch the straw with your hand.



## Unprocessed ejaculate

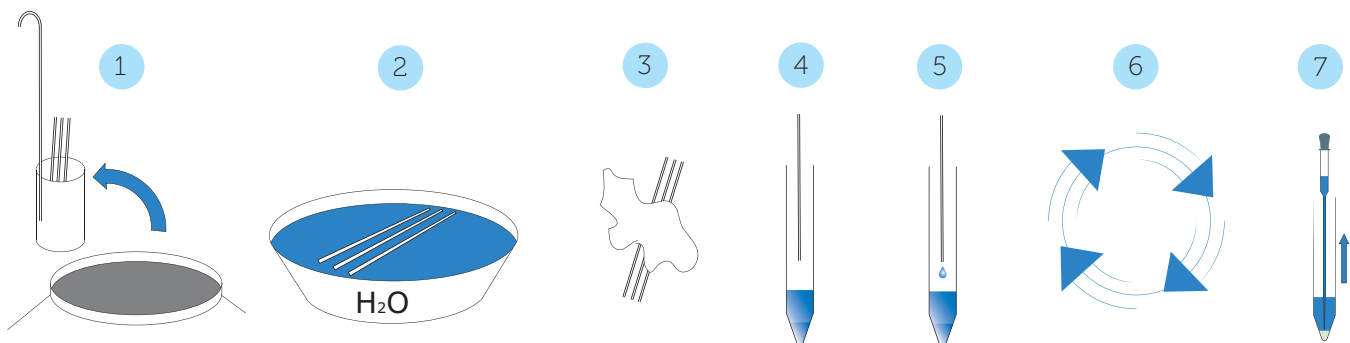
1. Measure the volume of the ejaculate.
2. Mix ejaculate with Sperm CryoProtect™, add 1 part of Sperm CryoProtect™ to 3 parts of sample (see table), ensuring thorough mixing after adding each drop slowly in order to avoid osmotic shock.
3. Transfer 0.8-1.8 ml of the mixture to 2 mL cryovials.
4. Equilibrate vials in refrigerator for 30-60 minutes.
5. Place the vials horizontally in nitrogen vapour, above the liquid nitrogen surface on a piece of styrofoam (CryoFloater™). Put on a lid and leave for 30 minutes.
6. Transfer the vials quickly into the liquid nitrogen and, thereafter, store in liquid nitrogen. Do not touch the vials with your hand.





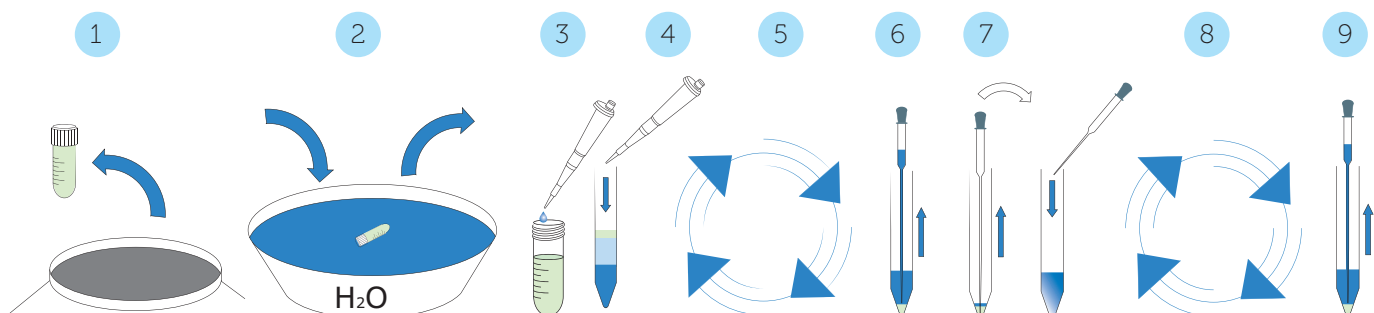
## Thawing procedure processed ejaculate

1. Remove the straws from the liquid nitrogen tank.
2. Place straws in water at 37°C for 30 secs.
3. Dry surface of straw.
4. Cut one end of straw.
5. Hold the straw over a tube with 5 mL PureSperm™ Wash and cut the other end of the straw. Any sperm suspension remaining in the straw can be expelled using a pipette.
6. Centrifuge at 500 x g for 10 minutes. Do not use the brake.
7. Aspirate PureSperm™ Wash supernatant leaving as much liquid as required for desired concentration. If no pellet is seen, leave the bottom 0.10 mL fluid.
8. The sample is now ready for use.



## Thawing procedure unprocessed ejaculate

1. Remove the vials from the liquid nitrogen tank.
2. Place vials in water at 37°C until all ice crystals are gone, approximately 2-3 min.
3. Dilute the thawed material with 0.5 ml PureSperm™ Wash.
4. Prepare the thawed material on a 40% and 80% PureSperm™ density gradient. Use 1 ml of each for the gradient and layer not more than 1 ml of the thawed ejaculate onto the gradient.
5. Centrifuge at 300 x g for 20 min.
6. Aspirate everything except the pellet and 4-6 mm of the PureSperm™ 80% layer.
7. Use a new pipette to aspirate the pellet. Transfer to a new tube containing 4 ml PureSperm™ Wash.
8. Centrifuge at 500 x g for 10 min.
9. Aspirate PureSperm™ Wash supernatant and the sample is now ready for use.



## SpermCatch™

### Background

SpermCatch™ is an alternative to PVP (polyvinylpyrrolidone) which today is the most common substance used for slowing down sperm prior to ICSI. However, PVP has been reported to cause problems, such as damaging the sperm plasma membrane. It may also interfere with sperm nucleus decondensation.

SpermCatch™ is a solution without PVP, contains instead hyaluronic acid which is a natural component. Several studies have shown that SpermCatch™ gives the same or even better results than PVP. Since SpermCatch™ is a solution containing hyaluronic acid, see the following reference for the advantages. (Ref 9-11, 22)

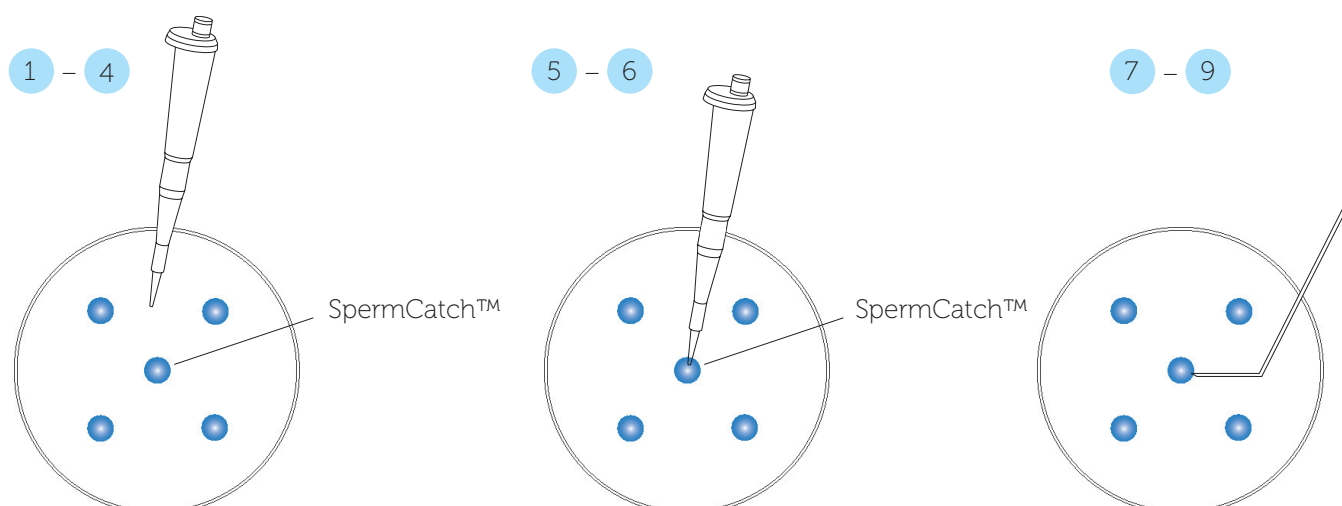
### Reagents and Equipment

SpermCatch™  
NidOil™  
Injection media

Sterile pipettes  
ICSI equipment  
Petri dish

### Procedure

1. Place a 10 µL drop of SpermCatch™ in the middle of a petri dish.
2. Place 4 drops of 10 µL injection media around the SpermCatch™ drop in the petri dish.
3. Immediately cover the drops with NidOil™.
4. Incubate for 30 minutes in CO<sub>2</sub> environment at 37°C.
5. Add 1 µL of prepared sperm suspension to the middle of the SpermCatch™ drop.
6. Incubate for 10 minutes in CO<sub>2</sub> environment at 37°C.
7. Fill your injection pipette with SpermCatch™ to avoid the sperm sticking to the inside of your pipette. It will also help you to make a controlled injection.
8. Immobilise the individual sperm by using the injection pipette to "nick" the sperm tail.
9. Aspirate the immobilised sperm.
10. Move to one of the oocyte droplets. Focus on the oocyte and position the oocyte with the holding pipette. Bring down your injection pipette and inject the sperm. Make sure that the oolemma is broken before you expel the sperm.



### Tips

- ICSI dishes must be prepared quickly to avoid osmolarity changes in the media. Only make two at a time.
- It is convenient to have two dishes per patient.

## NidOil™

## Background

Mineral oil to overlay the embryo culture is used extensively in IVF laboratories. NidOil™ is a paraffin oil which has been specifically chosen and then treated in our production facilities to ensure that its purity and handling characteristics are suitable for using as an overlay when culturing gametes and embryos.

NidOil™ does not require washing before use, and it is neither too sticky nor too viscous, to facilitate pipetting.

Our stringent quality assurance control helps main-

tain our standard for low endotoxin levels and also ensures our products are free from microbiological contamination.

There have been several reports of paraffin oils becoming embryo-toxic after exposure to light on the laboratory bench. As a precaution against any possible light-induced changes, NidOil™ is packaged in amber, screw-top bottles. (Ref. 13)

A prospective randomized study to compare four different mineral oils used to culture human embryos in IVF/ICSOI therapy (Ref. 25)

Presented at ESHRE 2008 by Dr C. Sifer, Paris

Comparison between;

1. Mineral Oil (CryoBioSystem)

3. Liquid Paraffin (ORIGIO)

3. NidOil (Nidacon)

4. Ovoil (Vitrolife)

Group	1	2	3	4
No of cycles	129	126	126	119
GQE day 3	2.1	1.6	2.2	2.6
Impl. rate %	22.1	21.7	30.0	24.6
Clin. Pregn %	31	29	38	36

## Recommendations before use

NidOil™ should be equilibrated in the same way as the culture medium before use to avoid differences in

temperature and gaseous content between the components of the culture system.

## Extra Quality Assurance test

Many questions have been raised lately to whether the oil that is used for covering an embryo culture can actually damage the embryo.

All oil batches today from different manufactures are tested for sterility, endotoxins and a mouse embryo assay showing blastocyst development. This is apparently not enough, since damage to cultures has been observed with an approved batch of oil.

One answer could be peroxidation of the oil which has been investigated in several publications (Ref. 27, 28) and found to be harmful to fertilisation and embryo development when over a certain

level. It has also been shown that the peroxidase level in oil can increase over time, due to exposure to light or high temperature storage.

The peroxidase test is now included in our Quality Assurance Certificate which comes with every batch and we also test the raw material before we make the order. In this way we hope to provide you with an oil for your cultures that is safe to use and still practical with the long shelf life and room temperature storage before opening.

If you have any questions regarding our tests, please contact us.

# Vitrification and warming of blastocysts

## VitriBlast™ Kit ThermoBlast™ Kit

### Background

The formation of intracellular ice crystals is a major problem during the cooling and warming of cells. These ice crystals have detrimental effects on cell survival rates. Vitrification, which is the rapid freezing of cellular material, makes it possible to freeze cells while, at the same, time avoiding the formation of intracellular ice crystals. The use of the vitrification technique results in a very homogeneous structure, an amorphous crystalline structure.

A vital feature of VitriBlast™ is the fact that DMSO and Ethyleneglycol are not included in the media, and needs to be added to the medium just before

use. DMSO and Ethyleneglycol are included as additives in the vitrification kit.

The reason for this is that DMSO oxidizes Human Serum Albumin (hSA) by the creation of di-sulfide bridges. This reaction is slow, but the longer the two substances are combined, the more bridges are created and dimers or oligomers of hSA would be created, thereby impairing its function. hSA could undergo considerable conformation changes and not effectively function as an excipient. To avoid this risk, DMSO is not included in the media, but supplied as an additive in the kit.

### Recommendations

VitriBlast™ is compatible with most devices. (i.e with all devices thus far tested; CryoLock, Cryo-Loop, and Rapid I). The most important factor is to use the type of device that feels safe and easy to use.

Work on a heated stage at all times when manipulating the blastocyst. Do not let the blastocyst remain exposed to the microscope light during incubation.

Collapsing the blastocyst will improve the results (Ref. 23). If laser is used, shoot as far from inner cell mass (ICM) as possible and ensure both the zona and the trophectoderm are breached.

If an ICSI-pipette or other sharp instrument is used, puncture right through the trophoblast cell layer into the blastocoele, and be sure to puncture as far as possible from the ICM.

The pipette should be inserted at the one o'clock position and exit through the blastocyst at the 11 o'clock position.

Collapsing is optional when vitrifying early blastocysts with smaller blastocoele cavities.

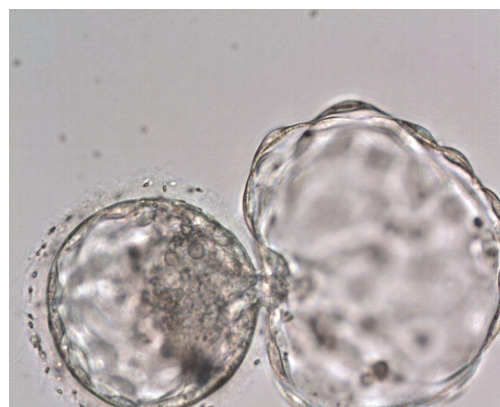
### Reagents and Equipment

VitriBlast™ and ThermoBlast™ Kit  
Sterile pipettes  
Device for vitrification  
CO<sub>2</sub> incubator  
Stopwatch

Liquid nitrogen reservoir  
Liquid nitrogen  
Culture dishes (NUNC 4-well)  
Heated stage  
Inverted microscope



Vitrified and warmed blastocyst with excellent morphology.

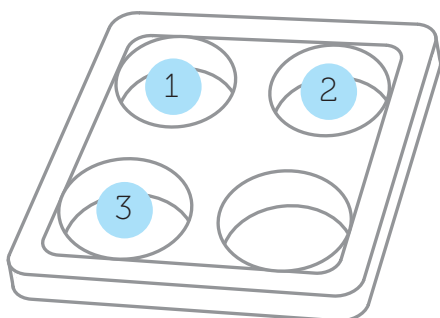


Hatching blastocyst after vitrification and warming.

## Vitrification procedure

1. Label the NUNC-dish with the patient ID and each well with corresponding solution number, i.e. 1, 2 and 3.
2. Pipette the vitrification media as described below. When adding the DMSO and Ethylene glycol (EG), which are included in the kit, to solutions 2 and 3, pipette the two up and down a few times to obtain optimal mixing of the media.

Well 1		Well 2	
VitriBlast™1	1000 µL	VitriBlast™2	850 µL
		DMSO:	75 µL
		EG:	75 µL
Well 3			
VitriBlast™3	700 µL		
DMSO:	150 µL		
EG:	150 µL		



3. Incubate at 37°C in 5-6% CO<sub>2</sub> for 30 minutes, maximum 1 hour.
4. During the 30 minute incubation of the dish, collapse the blastocyst. This can be done either by laser or by using an ICSI-pipette.

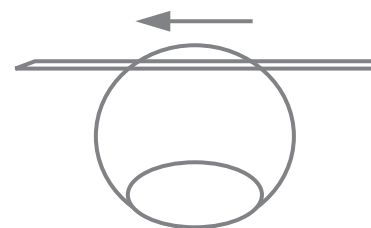
### Laser

- If laser is used, shoot as far from inner cell mass (ICM) as possible. The laser beam shoots vertically, aim as illustrated below.
- Be sure that you create a hole through the zona and the trophoctoderm.



### ICSI-pipette

- If an ICSI-pipette or other sharp instrument is used, puncture right through the trophoblast cell layer into the blastocoele, and be sure to puncture as far as possible from the ICM.
- The pipette should be inserted at the one o'clock position and exit through the blastocyst at the 11 o'clock position.



5. Remove the NUNC-dish from the incubator and place it on a heating stage (make sure the heat controller is set high enough to obtain 37°C in the media).
6. Place the punctured and collapsed blastocyst in solution 1. Start the stop watch.



# Vitrification and warming of blastocysts

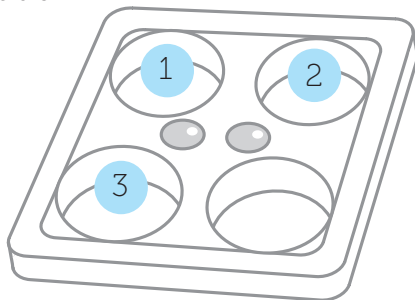
## Vitrification procedure

- After 1.5-2 minutes transfer the blastocyst by aspirating solution 2 into the pipette tip, collect the blastocyst from solution 1 and transfer it to solution 2.
- Incubate on the heating stage for EXACTLY 2 minutes. Start the stopwatch and observe when 2 minutes is approaching. It is easier to start the stopwatch and let it run towards 2 minutes. This removes the stress of the beeping noise when using a countdown timer. While incubating; proceed to step 9.
- At the end of 2 minutes, transfer the blastocyst by aspirating solution 3 from the well into the pipette tip, collect the blastocyst from solution 2, and transfer it to solution 3 in the 10  $\mu$ L droplet. The blastocyst must remain in solution 3 for 30–45 seconds, including the time on the device.
- Place the blastocyst on the device.
- Immerse in liquid nitrogen.



*Do not let the blastocyst remain exposed to the microscope light during the incubation.*

- During the 2 minute incubation, prepare 2 x 10  $\mu$ L drops of solution 3 in the middle of the dish (see diagram below). The droplets evaporate quickly. It is better to prepare them as late as possible.



- Store in liquid nitrogen.

## Tips

- If the additives are stored in the refrigerator, remove them in good time prior to use. DMSO turns solid below +18°C. The additives can be stored outside the refrigerator in the supplied packaging even after opening. If urgent the DMSO bottle can be warmed by holding the bottle in your hand.
- The EG and DMSO can be pre-mixed in the bottles (VB 2 and VB 3) and stored in the refrigerator for up to a week. However it is important to know that the volumes in the VB 2 and 3 are not EXACTLY 10 mL, there is always a little surplus. Therefore, the volumes in the VB 2 and 3, must be measured and the surplus discarded prior to adding the EG and DMSO in order to achieve the correct concentrations.
- Using drops reduces the risk of losing the blastocyst. The blastocyst tends to float on the viscous solution 3. It is also important to incubate solution 3 under the same conditions as the other two solutions, hence the use of 1 mL.

## Warming procedure

1. Label the NUNC-dish with the patient ID and each respective well with each solution number, i.e. 4, 5, 6.
2. Pipette the warming media 4, 5 and 6 as described below.

Well 1

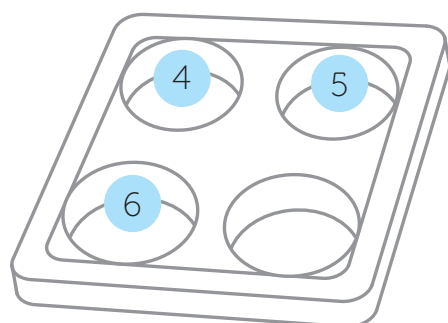
ThermoBlast™4: 1000 µL

Well 2

ThermoBlast™5: 1000 µL

Well 3

ThermoBlast™6: 1000 µL



3. Incubate at 37°C in 5-6% CO<sub>2</sub> for 30 minutes.

4. Immerse only the device in the surface of solution 4. Let the blastocyst fall off. Identify its presence in the well and incubate for 2 minutes on the heating stage. (2 minutes includes time for "locating" the blastocyst).
5. Transfer the blastocyst to solution 5. Let the blastocyst simply sink to the bottom, do not wash. Incubate for 3 minutes in solution 5.
6. Transfer the blastocyst to solution 6.
7. Incubate for 5 minutes.
8. Transfer the blastocyst to culture medium and allow the blastocyst time to reexpand. Wait 1 to 4 hours before final assessment. If the blastocyst has not reexpanded after 4 hours the chance of reexpansion is low.

## Tips

- Do not aspirate solution from TB5 before aspirating from TB4. This is to allow the blastocyst to sink in TB5 which makes it easier to identify. Do not aspirate solution from TB6 when aspirating from TB5. This is to allow the blastocyst to sink in TB6 which again makes it easier to identify.

# Viability Staining

## Sperm VitalStain™

### Background

Sperm vitality should be determined in semen samples with 50% or more immotile spermatozoa according to the WHO laboratory manual for the examination of human sperm.

SpermVitalStain™ uses the eosin-nigrosine technique in a one-step method to establish the

percentage of live spermatozoa. It is based on the principle that dead cells (i.e. those with damaged plasma membranes) will take up the eosin and stain red. Nigrosine provides the background to facilitate visualisation of the unstained (white) live cells. (Ref. 12)

### Reagents and Equipment

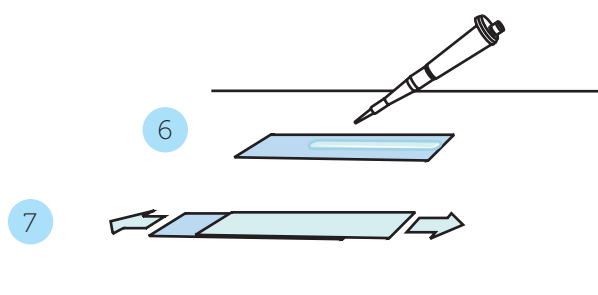
Light microscope (40 – 100 x magnification)  
Microscope slides

Pipette  
Test tube

### Procedure

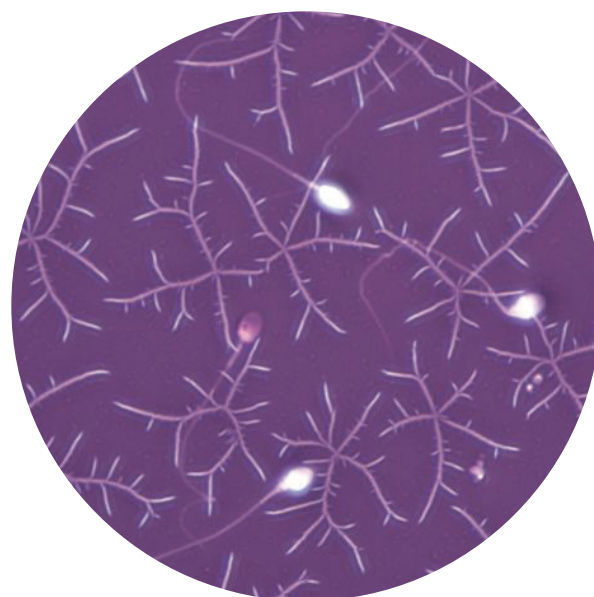
1. Shake the bottle of Sperm VitalStain™ before use.
2. Take an equal amount of Sperm VitalStain™ and the sperm sample (eg. 50 µL SVS + 50 µL sample). Use for example an eppendorf tube.
3. Mix well.
4. Leave for 30 seconds at room temperature.
5. Prepare a slide using your conventional method or use the method recommended by Nidacon.
6. Transfer a 20 µL drop onto a labelled microscope slide with a pipette, making a string/line of fluid in the middle of the slide.
7. Cover this slide with a second microscope slide and, when the drop is evenly spread between

- the two slides, pull them apart from each other horizontally. You now have two good slides.
8. Air dry the two slides and examine. If you want to store for later use, mount the slides with DPX or equivalent mountant, and a cover slide.
9. Examine using a bright field 40 x objective or a 100 x objective under oil immersion.
10. Count 200 sperm, the white (unstained) are classified as alive and the red or pink are classified as dead. Sperm coloured only at the neck region are classified as alive.



### Tips

- The 100x objective with immersion oil will give you a very clear picture of stained versus unstained sperm.



## References

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