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Spermac Stain

Staining kit for human spermatozoa

For in vitro diagnostic use
Reagents for professional use only

IVD

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MATERIAL INCLUDED

Catalogue number

SPS050	4x 50ml	Spermac Stain
SPS250	4x 250ml	Spermac Stain

CUSTOMER-TECHNICAL SUPPORT

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Spermac Stain

GENERAL INFORMATION

Sperm morphology analysis is one of the basic semen examinations performed in the diagnosis and management of male infertility. Spermac Stain is an in vitro diagnostic staining kit consisting of a fixative and 3 staining solutions for human spermatozoa. Staining facilitates distinction between morphologically normal and abnormal spermatozoa, and enhances visualization of different parts of the sperm cell (head, acrosome, equatorial region, midpiece, tail) [1,2].

INTENDED USE

Spermac Stain is a qualitative, non-automated, diagnostic kit for professional use for the staining of human spermatozoa. The purpose of staining spermatozoa is to facilitate differentiation between morphologically normal and abnormal spermatozoa. The outcome of this evaluation may help in assessing the diagnosis and management of male infertility.

MATERIAL INCLUDED IN THE KIT

Spermac Stain

Product code:	**SPS050**	**SPS250**
Stain A: Red stain	50 ml	250 ml
Stain B: Pale green stain	50 ml	250 ml
Stain C: Dark green stain	50 ml	250 ml
Fix: Fixative	50 ml	250 ml

The certificate of analysis and MSDS are available on request or can be downloaded from our website (www.fertipro.com).

MATERIAL REQUIRED, BUT NOT PROVIDED

- Microscope slides
- 5 Coplin jars
- Microscope (1000x magnification)
- Immersion oil
- Warm plate (37 °C)
- Tap or distilled water

METHOD

Scan barcode (or follow link on www.fertipro.com) to view the demonstration video.

SPECIMEN COLLECTION AND PREPARATION

The abstinence period should be 2-7 days. Avoid the loss of the first semen fraction as this contains proportionally more normal spermatozoa. Do not wait more than 4 hours after ejaculation before starting the test.

REAGENT PREPARATION

1. Pour Stain A, B and C in separate Coplin jars, make sure the fluid level is high enough to cover the area that is to be stained.
2. Fill a Coplin jar or cradle with tap water for the wash steps (see Note 1)
3. Prepare glass slides: clean, wash in alcohol and let them dry
4. Keep Fixative reagent bottle closed ! (see Note 2)

Note 1: use distilled water if tap water is alkaline (pH > 7). If a cradle is used that allows combination of multiple slides, ensure that it is large enough to ensure complete washing.
Note 2: Fixative vapor interferes with the staining, even in very small amounts.

STAINING PROCEDURE

1. Mix the semen well to have a homogenous sample and prepare a thin feathered-edge smear of fresh, undiluted, preferably liquified semen on a glass slide (e.g. 10µl semen). Allow the smear to air dry for about 5 minutes on a warm plate at 37 °C.
2. When the smear is dried, pour Fixative in a jar. Perform each handling with Fixative **under a fume hood!**
   a. Fix the smear by immersing the slide for minimum 5 minutes in the Fixative jar. Longer fixation is acceptable but not necessary.
   b. Remove slide from the Fixative jar, briefly place vertically on absorbent paper to drain excess of fluid. Do not touch the specimen with the paper.
   c. Let the slide dry by placing it on a warm plate at 37 °C for 15 minutes. Meanwhile, remove Coplin jar with Fixative from the work area.
3. Wash by gently dipping 7x in the water jar (see Note 1 above). If needed (e.g. when using a small Coplin jar), repeat the washing procedure with fresh water to ensure complete washing. Briefly drain excess water off by touching the end of the slide onto absorbent paper.
4. Slowly dip slide 7x in and out of Stain A (see Note 3). Then leave undisturbed for 2 minutes in the jar. Afterwards, place vertically on absorbent paper. Wash in fresh water and drain as specified in step 3. Repeat the washing in fresh water. **Double washing after Stain A is important**.
5. Dip slide 7x in and out of Stain B. Then leave undisturbed for 1 minute in the jar. Afterwards, place vertically on absorbent paper. Wash in fresh water and drain as specified in step 3.
6. Dip slide 7x in and out of Stain C. Then leave undisturbed for 1 minute in the jar. Afterwards, place vertically on absorbent paper. Wash in fresh water and drain as specified in step 3.
7. Allow slide to air dry.
8. Observe slide under a light microscope (1000x) using oil immersion.

Note 3: 'slowly' means: about 1 dip per second. Dipping is important because it ensures complete contact of the sample with the stain.

INTERPRETATION

- acrosome = dark green
- nucleus = stained red
- equatorial region = pale green
- midpiece and tail = green

- Count at least 100 and preferably 200 spermatozoa and classify them as either normal or abnormal, specifying which defects are most common.
- Only include identifiable sperm cells in the count.
- Using the 2021 WHO criteria, a sample is considered normal if at least 4% of spermatozoa show normal forms[3].

By the strict application of certain criteria of sperm morphology, relationships between the percentage normal forms and various fertility endpoints (time-to-pregnancy, pregnancy rates in vivo and in vitro) have been established, which may be useful for the prognosis of fertility[3].

REMARKS ON USE

- Proteinaceous or gelatinous samples and frozen samples must be diluted 1:1 with 3% sodium citrate prior to smearing.
- A stained slide should be transparent with only a very slight hint of green hue. If the slide is dark green, then the slide was exposed to fixative vapors before fixing.
- For transport prior to staining, slides may be prepared, fixed, washed, and dried. Protect against abrasion during transport. When ready to stain, begin the process at the fixative (Step 2), i.e. the slides receive a double fixation. This is important as the fixative contains buffers that ensure that subsequent staining occurs correctly.

MOUNTING SLIDES

Staining will fade under mounting medium (after several weeks). Therefore, do not mount slides if you want to use it as a reference later on. Gently blot off immersion oil, which also causes fading. It is preferable to make duplicate slides for future reference if necessary, or photographic and/or video records.

LIMITATIONS OF THE METHOD

Spermatozoa stained with Spermac Stain cannot be used for any other procedure.

STORAGE / DISPOSAL AND STABILITY

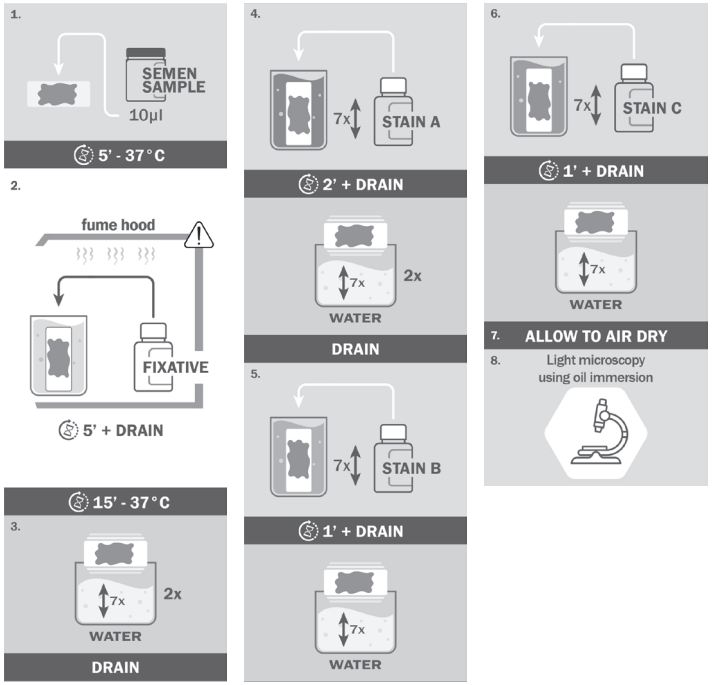
- Spermac Stain should be stored in closed Coplin jars or the original bottles, at 2-25 °C.
- Suitable for transport or short term storage at elevated temperatures (up to 5 days at 37 °C).
- The reagents are stable until the expiry date specified on the label. Do not use after expiry date.
- However, staining removes constituents and introduces contaminants, and thus stains should be replaced when adequate staining is no longer achieved.
- Filter stains if deposit is noted.
- The reagents need to be disposed in accordance with local regulations for disposal of medical devices.
- Number of tests that can be performed with one Spermac Stain kit is difficult to determine since stains can be reused.

WARNINGS AND PRECAUTIONS

- All human, organic material should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or Hepatitis. Always wear protective clothing when handling specimens and reagents (gloves, lab vest, eye/face protection).
- Fix: contains paraformaldehyde; may cause an allergic skin reaction; causes serious eye irritation; suspected of causing cancer.
- Due to toxicity when inhaling paraformaldehyde, steps using the fixative should be performed under a fume hood.
- Stain A and Stain B: highly flammable liquid and vapor
- The Stains contain substances which are identified as mutagenic. However, as the concentration of these substances in the final reagent is low, the Stains itself are not identified as mutagenic.
- The kit does not contain any endocrine disrupting substances.

REFERENCES

[1] Oettlé EE(1986). An improved staining technique which facilitates sequential monitoring of the acrosome state, Development, Growth and Differentiation (Suppl.): 28
[2] Chan PJ, Corselli JU, Jacobson JD, Patton WC, King A (1999). Spermac stain analysis of human sperm acrosomes. Fertility and Sterility 72 (1): 124-128.
[3] Geneva: World Health Organization. 2021. 'WHO Laboratory manual for the examination and processing of human semen', sixth edition.



SYMBOLS GLOSSARY

Symbols as defined in ISO 15223

REF	Catalogue number	LOT	Batch code
	Consult instructions for use		Manufacturer
IVD	In Vitro Diagnostics	2°C 25°C	Temperature limit
	Use-by date		

Symbol as defined in IVDR 2017/746

CE	CE marking

Symbol as defined in Regulation (EC) No. 1272/2008 [CLP]

	GHS07: Health hazard: may cause an allergic skin reaction		GHS08: Health hazard: suspected of causing genetic defects, may cause cancer
	GHS02: Flammable: highly flammable liquid and vapor		