



SpermBlue

kit for sperm morphology assessment

SpermBlue Staining Procedure

KIT FOR SPERM MORPHOLOGY ASSESSMENT IN HUMAN AND ANIMAL SPECIES.

Background:

The stain has been developed to stain all components of sperm (acrosome, head, midpiece, principle piece of tail and end piece) differentially in different intensities of blue. The staining procedure is very simple and only involves two main steps, fixing in one medium and staining in a second medium. It works equally well for smears of “raw” semen as well as swim-up/Percoll/PureSperm gradient preparations, using most tissue culture media.

Please note that only fixed/dead sperm to be stained. Not for use with live unfixed cells in any procedure such as in tissue culture.

Contents of SpermBlue®:

All SpermBlue® packages contain bottles/staining trays with equal volumes of fixative (clear or transparent solution marked Fix) and a dark blue stain (dark blue staining solution marked Stain).

SpermBlue® is packaged in two bottles, individually containing 250 ml fixative and 250 ml stain. Sufficient to stain 600 sperm smears or more on slides.

It is recommended that the fixing and staining of smears are performed in standardized containers, e.g. plastic Coplin jars, which could be provided on request.

If fixative and stains are stored at 4°C it will last for at least one year or longer. Room temperature storage (20 – 25°C) not guaranteed but normally lasts one year. Take note of expiry date.

Staining Procedure:

1. Make duplicate sperm smears using 10µl of semen or 10 to 15µl of swim-up sperm (adapt volume to concentration of sperm) and allow to air dry. If sperm concentration in semen is less than 20 million/ml, use 15µl of semen for smear. Ideal angle of slide which is used to make smear is about 45°. If sperm concentration is low, decrease angle of slide which is used to make smear to about 20°. A larger volume of sperm will accordingly be dragged behind moving slide resulting in more sperm on slide. Ensure sperm smear is totally dry before next step.

2. Carefully place dried smears vertically into staining tray (Coplin-type jar) containing SpermBlue® fixative. Take care to slowly immerse slides into fixative and leave – no agitation, etc. Fix for 10 minutes at 20 to 25°C.

Alternatively, place dried smears horizontally down on filter paper (smear up). Use a plastic disposable pipette to put 0.5 to 1ml of clear fixative solution on dried smear, and make sure to cover the whole area of the smear. Leave for 10 min – do not roll from side to side.

3. Carefully remove slides from staining tray and hold it at an angle of 60° to 80° to drain off excess fixative. If smears were fixed horizontally, let most of fixative gently run off slide onto filter paper. No washing or drying is needed after fixation.

- Carefully place fixed smears vertically into staining tray containing SpermBlue® stain. Take care to slowly immerse slides into stain and leave – no shaking or moving of slide in medium. Stain for 12-15 minutes at 20 to 25°C.

Alternatively, place slides horizontally down onto filter paper after fixation. Use a plastic disposable pipette to put 0.45 to 0.5ml of stain onto fixed sperm smear. Gently roll slide from side to side at regular intervals (once every minute) to ensure that stain is displaced equally across smear surface. Stain for 12-15 minutes.

- Carefully remove slides from staining tray. If smears were stained horizontally, let most of stain gently run off slide onto filter paper. SLOWLY dip stained slides into distilled water – only one dip, lasting for 3 seconds is required. Immersion technique usually requires only one dip to wash off excess stain, but second dip might be necessary for the “drop-on” technique. Slides need to be very gently dipped in distilled water, preventing too many sperm from being lost during this washing step. Remove slides from water very slowly and leave in an upright position (at about 70° angle). Let all fluid drain from slide until air dry.
- Mount slide with DPX or equivalent synthetic medium for making permanent slides. When the mounting medium is dry view under oil immersion x1000 for human sperm.
- If nuclear staining is not intense enough, stain the duplicate smear for another three to five minutes and even longer if required.

Alternative fixation procedure:

In some instances 95% ethanol or 100% methanol fixation (coagulant fixation) may be used particularly after washing sperm and to concentrate sperm instead of SpermBlue® fixation. Staining procedure after alcohol fixation in SpermBlue® remains the same as outlined above.

Important comments:

Initial staining results may suggest either too little staining of some sperm as well as differences in staining intensity on the same slide. Each researcher has to experiment to optimize her/his results in this context. Try and adapt staining times at temperature conditions between 20 and 30°C.

Many existing sperm staining techniques rely on “sperm painting” which is not cytologically acceptable. SpermBlue® clearly differentiates all sub-divisions of sperm accurately and is particularly good in the identification of the sperm acrosome (van der Horst and Maree, (2009) SpermBlue®: A new universal stain for human and animal sperm which is also amenable to automated sperm morphology analysis, Biotechnique and Histochemistry: In press).

Examples:

With human and sub-human primate sperm the acrosome stains light blue and the head dark blue. Midpiece stains distinctly dark blue, rest of tail slightly lighter blue and end piece even lighter blue. Suitable for SCA automatic Morphology analysis (Microptic SL, Barcelona, Spain).

In domestic animals such as bull, boar and ram: Acrosome stains dark blue, post acrosomal area and particularly the equatorial zone stains light blue. Midpiece stains darker blue and rest of tail slightly less dark blue.

Safety data sheet for SpermBlue®:

SpermBlue® contains toxic components like all cytological stains but is not hazardous. The main active component is a slight skin, oral/nasal irritant and staining should preferably take place in a fume hood. If skin contact has occurred, wash affected area thoroughly with water.

Precautions:

All cytological stains are toxic and have to be handled with care. Always work with gloves and preferably in a fume cupboard. Only stain when sperm are fixed (dead). Do NOT use for live unfixed cells.

Distributed by:

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