DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Moti-sperm technical information
Technical card code 13-100
Product code 13-100
Pack 4x50 ml or on request
Stability of product properly conserved at 15-20°C 24 months
CND code W01030799

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For emergency contact your nearest anti-poison unit.

Principle. Moti-sperm is an IVD kit for staining human spermatozoa. The purpose of staining spermatozoa is to be able to differentiate morphologically normal from abnormal sperm cells. The definition and criteria for normality have been largely based on studies done on sperm recovered from the female reproductive tract (especially in post coital cervical mucus) which is considered to be normal. Still different criteria have been proposed, the main ones being the WHO criteria (see WHO laboratory manual for the examination and processing of human semen, 5th edition, WHO, 2010). Moti-sperm stain is an aid in evaluating morphology as it helps distinguish the different parts of the sperm cell (head, acrosome, equatorial region, mid-piece, tail), making it easier to differentiate between a normal and an abnormal spermatozoon. Moti-sperm may help in assessing the diagnosis and the management of male infertility.

Method. Pour the reagents in coplin jars, make sure the fluid level is high enough to cover the area that is to be stained. Only fill the fixative jar when the slides have been prepared, dried, and are ready for staining. Fill a fifth coplin jar, or any other recipient that can contain a complete object glass, with tap water (for washing the slides between the different dyes). Always use distilled water for washing. Clean, wash in alcohol and dry slides before use.

1) Allow a thin feathered-edge smear of fresh, undiluted semen to air dry for five minutes on a warm plate at 37°C.
Note.
Do not make or dry smears close to the open bottle of fixative, as the fixative vapour (even in very small amounts) interferes with the staining.
2) Fix the smear by immersing the slide for a minimum of five minutes in a copling containing the fixative. Longer fixation is acceptable but not necessary.
3) Remove slide from fixative, briefly place vertically on absorbent paper to drain excess fixative. Do not touch the specimen with the paper.
4) Wash by gently dipping seven times in distilled water. If slides are stained in a cradle containing five or more slides, ensure that the washing container is large enough to ensure complete washing of the fixative off the slides. If the washing container is small then repeat the washing procedure with fresh water. Briefly drain excess water off by touching end of slide onto absorbent paper.
5) Stain in solution (1), one minute.
When introducing the slide into the stain solution, dip slide seven times slowly (about one dip per second) in and out of the stain, to ensure complete contact of the sample with the stain. Then leave undisturbed for the rest of the staining period. Wash by dipping seven times in distilled water.
Briefly drain excess water onto absorbent paper. Repeat the washing in distilled water. This double washing step after Stain (1) is important. Briefly drain off excess water onto absorbent paper.
6) Stain one minute in solution (2). Dip seven times initially to ensure complete contact of stain with the specimen. Wash as above in distilled water.
7) Stain one minute in solution (3), dipping seven times initially. Wash in distilled water.
8) Allow smear to air dry.
9) Observe staining under a light microscope (1000x) using oil immersion:

Mounting Slides
If slides are mounted staining will fade under mounting medium (after weeks). So do not mount slides if you want to refer back later. Gently blot off immersion oil, which also fades the staining. It is preferable to make duplicate slides for future reference if necessary, or photographic or video records.

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Result

Acrosome	dark green
Nucleus	stained red
Equatorial region	pale green
Midpiece and tail	green

Interpretation
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. The criteria for classifying sperm cells as either normal or abnormal depends on the classification method used in the lab (WHO, 2010). According to the WHO, using 2010 WHO criteria, a sample is considered normal if at least 4% of spermatozoa show normal forms. 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 (WHO, 2010).

Reagent

Fixative	50 ml
Solution (1)	50 ml
Solution (2)	50 ml
Solution (3)	50 ml

Storage and stability
Moti-sperm stain should be stored in closed jars or the original bottles, at 15-25°C. The reagents are stable for 24 months after date of manufacture if unused. 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.

Warnings. All semen samples should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or Hepatitis.

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 vapours 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.

References
WHO laboratory manual for the examination and processing of human semen, 5th edition, WHO, 2010