FluoVit Protocol

VITALITY ASSESSMENT UNDER FLUORESCENCE FOR HUMAN AND MOST ANIMAL SPECIES

The assessment of sperm vitality is one of the basic steps for the semen analysis.

FluoVit is a very useful fluorescent staining solution that permits to differentiate living from dead spermatozoa (**RED**=dead, **BLUE**=alive). This kit is composed of two fluorescent dyes and must be used with fluorescence microscopy.

FluoVit is intended for use as evaluation of sperm vitality in routine assessment of male infertility or research studies in animal.

The kit can be used with fresh or thawed semen samples.

200 µl BLUE + 200 µl RED = 200 analyses

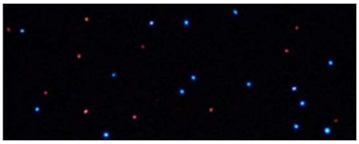
Storing conditions: solutions should be stored at 2-4°C protected from light.

Caution: the stain solution is mutagen and must be handled with care. The dye must be disposed of safely and in accordance with applicable regulation.

High sensitivity: the dye detects low levels of nucleic acid in sperm.

Reagents for in vitro diagnostic use only: The **FluoVit** includes Hoechst 33342, Trihydrochloride Trihydrate (330/380) and Propidium Iodide (536/617) based solutions stabilized for long lasting.

Microscope filter: **DAPI filter** (EX 330-380, DM 400, BA 420, standard filter for UV).



Procedure:

Step 1: put 10 µl of semen sample in an empty vial and add 1 µl of **BLUE** Eppendorf stain (Hoechst and trihydrochloride trihydrate) previously heated at 37°C (we recommend to heat only the quantity of solution that is going to be used for the analysis in a different vial, in order not to damage the rest of the fluorochrome solution with temperature shocks). Ensure that the semen **sample has a maximum concentration** of 50M/ml and make the appropriate dilutions with PBS. Otherwise, the concentration is too high to distinguish individual cells.

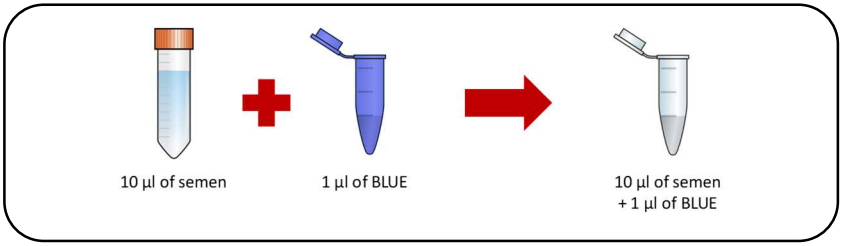
Step 2: leave the Eppendorf in the incubator at 37°C or room temperature for 5 minutes.

Step 3: take the vial from the incubator and add 1 µl of **RED** Eppendorf stain (Propidium Iodide) previously heated at 37°C (we recommend to heat only the quantity of solution that is going to be used for the analysis in a different vial, in order not to damage the rest of the fluorochrome with temperature shocks); mix gently with the micropipette. Wait 5 minutes (or until spermatozoa is detected).

Step 4: put an aliquot of 5 to 10 µl of stained sample on a standard slide with cover glass and analyze it under fluorescence microscopy.

Most animal species samples will be properly stained using the above procedure. In case of difficulties with the sample a working solution must be prepared (See page 3 of the current protocol).

Step 1:



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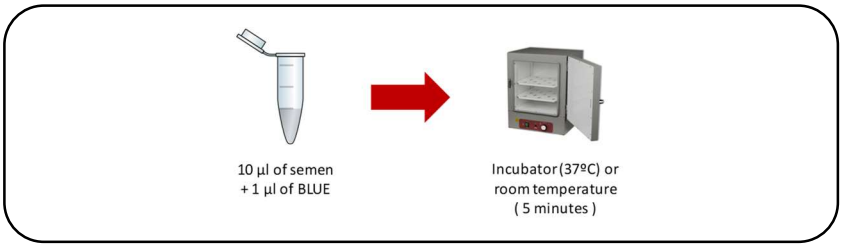

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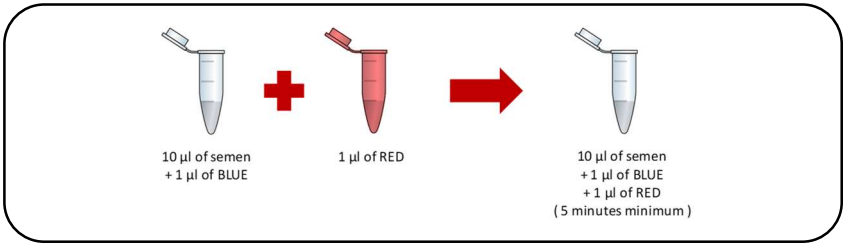

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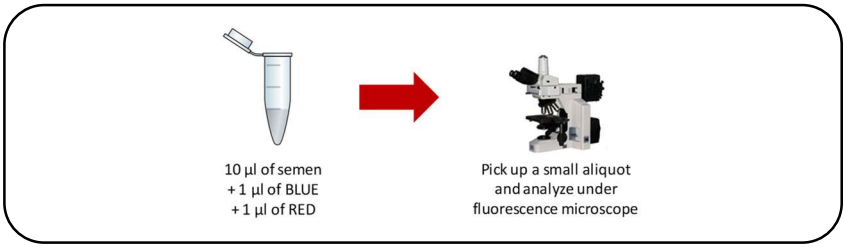


FluoVit Protocol

VITALITY ASSESSMENT FOR RAT, DOG AND OTHERS

The assessment of sperm vitality is one of the basic steps for the semen analysis.

FluoVit is a very useful fluorescent staining solution that permits to differentiate living from dead spermatozoa (**RED**=dead, **BLUE**=alive). This kit is composed of two fluorescent dyes and must be used with fluorescence microscopy.

FluoVit is intended for use as evaluation of sperm vitality in routine assessment or research studies.

The kit can be used with fresh or thawed semen samples.

200 µl BLUE + 200 µl RED = 200 analyses

Storing conditions: solutions should be stored at 2-4°C protected from light.

Caution: the stain solution is mutagen and must be handled with care. The dye must be disposed of safely and in accordance with applicable regulation.

High sensitivity: the dye detects low levels of nucleic acid in sperm.

Reagents for in vitro diagnostic use only: the **FluoVit** includes Hoechst 33342, Trihydrochloride Trihydrate (330/380) and Propidium Iodide (536/617) based solutions stabilized for long lasting.

Microscope filter: **DAPI filter** (EX 330-380, DM 400, BA 420, standard filter for UV).

For sperm of rat, dog and other animal species that are difficult to stain with the standard FluoVit procedure a **working solution** must be prepared.

Preparation of the working solutions:

Take 2 µl of **BLUE** Eppendorf and put them in a vial. Next add 998 µl of HBSS (Hank's Balanced Salt Solution) or other.

Step 1: *Use the BLUE working solution*

Repeat the procedure with the **RED** Eppendorf, taking 2 µl of the solution provided and adding 998 µl of HBSS or other buffer/media in a vial.

Use these working solutions to evaluate rat and other animal species that are not properly stained with the commercial solutions provided and follow the standard procedure.

These solutions are only for the day of the evaluation and **cannot be stored**.

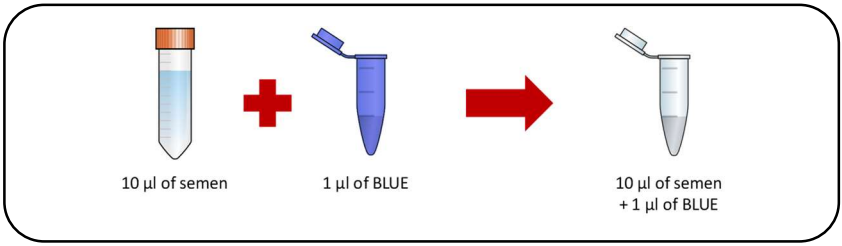
Procedure:

Step 1: put 10 µl of semen sample in an empty vial and add 1 µl of **BLUE working solution** prepared (Hoechst and trihydrochloride trihydrate) and previously heated at 37°C (we recommend to heat only the quantity of solution that is going to be used for the analysis in a different vial, in order not to damage the rest of the working solution with temperature shocks). Ensure that the semen **sample has a maximum concentration** of 50M/ml and make the appropriate dilutions with PBS. Otherwise, the concentration is too high to distinguish individual cells.

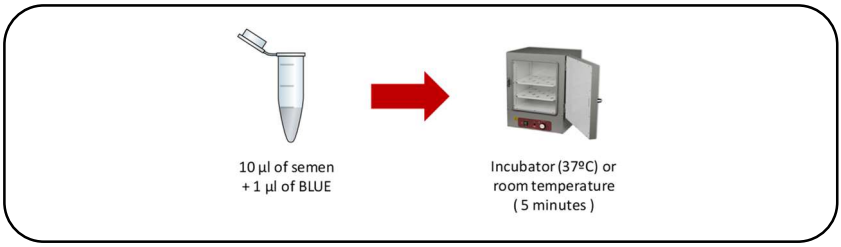
Step 2: leave the Eppendorf in the incubator at 37°C or room temperature for 5 minutes.

Step 3: take the vial from the incubator and add 1 µl of **RED working solution** previously prepared (Propidium Iodide) previously heated at 37°C (we recommend to heat only the quantity of solution that is going to be used for the analysis in a different vial, in order not to damage the rest of the working solution with temperature shocks); mix gently with the micropipette. Wait 5 minutes (or until spermatozoa is detected).

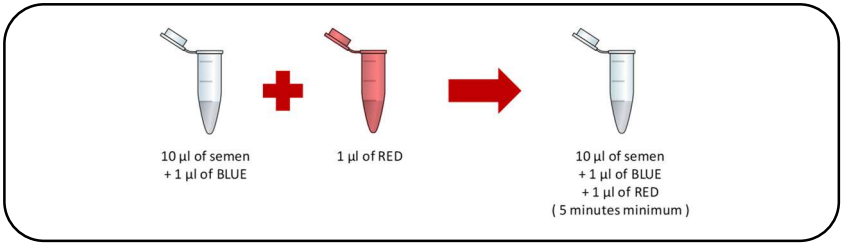
Step 4: put an aliquot of 5 to 10 µl of stained sample on a standard slide with cover glass and analyze it under fluorescence microscopy.



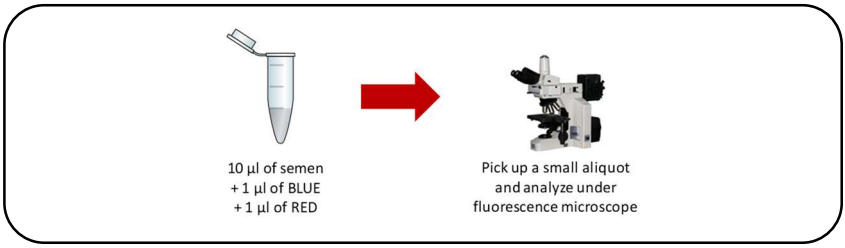
Step 2:



Step 3: *Use the RED working solution*



Step 4:



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