The assessment of sperm vitality is one of the basic elements for the semen analysis. **FluoVit** sperm is a very useful fluorescent staining solution that permits to differentiate living from dead spermatozoa (RED=dead, BLUE=alive). This kit is composed by 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 is can be used with fresh or thawed semen samples.

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**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 vial 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.

**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).

![FluoVit Image](image.png)
Step 1:

10 μl of semen + 1 μl of BLUE → 10 μl of semen + 1 μl of BLUE

Step 2:

10 μl of semen + 1 μl of BLUE → Incubator (37°C) or room temperature (5 minutes)

Step 3:

10 μl of semen + 1 μl of BLUE + 1 μl of RED → 10 μl of semen + 1 μl of BLUE + 1 μl of RED (5 minutes minimum)

Step 4:

10 μl of semen + 1 μl of BLUE + 1 μl of RED → Pick up a small aliquot and analyze under fluorescence microscope
FluoVit Protocol

VITALITY ASSESSMENT FOR RAT, DOG AND OTHERS

The assessment of sperm vitality is one of the basic elements for the semen analysis. FluoVit sperm is a very useful fluorescent staining solution that permits to differentiate living from dead spermatozoa (RED=dead, BLUE=alive). This kit is composed by 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 is can be used with fresh or thawed semen samples.

\[
200 \mu l \text{ BLUE} + 200 \mu l \text{ RED} = 200 \text{ analyses}
\]

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.


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.

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 1: *Use the BLUE working solution*

![Image](89x469 to 338x541)

10 μl of semen + 1 μl of BLUE ➔ 10 μl of semen + 1 μl of BLUE

Step 2:

![Image](134x353 to 291x425)

10 μl of semen + 1 μl of BLUE ➔ Incubator (37°C) or room temperature (5 minutes)

Step 3: *Use the RED working solution*

![Image](106x237 to 321x309)

10 μl of semen + 1 μl of BLUE + 1 μl of RED (5 minutes minimum)

Step 4:

![Image](129x121 to 298x194)

10 μl of semen + 1 μl of BLUE + 1 μl of RED ➔ Pick up a small aliquot and analyze under fluorescence microscope

Distributed by:

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