FluoVit

kit for sperm vitality assessment

FluoVit Protocol

VITALITY ASSESSMENT UNDER FLUORESCENCE IN HUMAN AND ANIMAL SPECIES.

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 live from dead sperm (RED=dead, BLUE=alive) This kit is composed by two fluorescent dyes and may be used with fluorescence microscopy.

The **FluoVit** sperm kit is for the evaluation of sperm vitality in fresh or unthawed semen samples. The kit is intended for standard semen analysis in routine assessment or research studies of male fertility or anomal.

200 µl BLUE + 200 µl RED = 200 analyses

Main characteristics of FluoVit

Storing conditions: Solutions should be stored at 2-6° degrees protected from light.

Caution: The stain solution is mutagen and should 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.

Fluorescent spectral characteristics: The **FluoVit** includes Trihydrochloride Trihydrate (330/380) and Propidium Iodide (536/617) based solutions stabilized for long lasting.

The microscope filter must be DAPI filter (EX 330-380, DM 400, BA 420, standard filter for UV).

High resolution: High resolution images with higher contrast.

Mode of use:

Step 1: Put 10 µl of semen sample in a empty eppendorf and add 1 µl of **BLUE** eppendorf stain (trihydrochloride trihydrate) previously heated at 37ºC (we recommend to heat only the numbers of microliters that are going to be used for the analysis of the day in a different vial in order not to damage the rest of the fluorochrome with temperature changes).

Step 2: Leave the eppendorf in the incubator at 37ºC or room temperature for 5 minutes.

Step 3: Take off the eppendorf from the incubator and add 1 µl of **RED** eppendorf stain (propidium Iodide) previously heated at 37ºC (we recommend to heat only the numbers of microliters that are going to be used for the analysis of the day in a different vial in order not to damage the rest of the fluorochrome with temperature changes); mix gently with the micropipette. Wait 5 minutes (or until BLUE sperm could be detected correctly).

Step 4: Put a small aliquot of 10 µl of stained sample on a standard slide, cover with cover glass and analyze under fluorescence.

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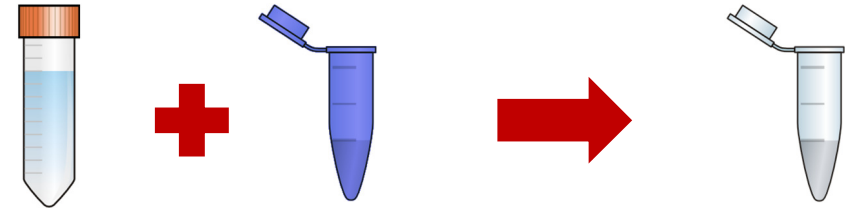
MICROPTIC S.L.
Viladomat, 321 – 6º 4ª
08029 Barcelona (Spain)

Tel: +34 93 419 29 10
Fax: +34 93 419 94 26

www.micropticsl.com
micropticsl@micropticsl.com

Step 1:

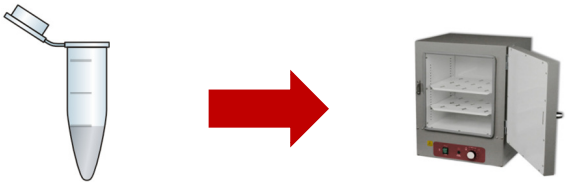


10 µl of semen

1 µl of BLUE

10 µl of semen
+ 1 µl of BLUE

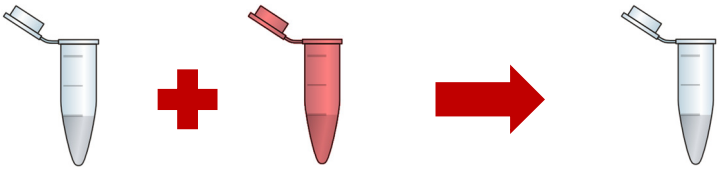
Step 2:



10 µl of semen
+ 1 µl of BLUE

Incubator (37ºC) or
room temperature
(5 minutes)

Step 3:

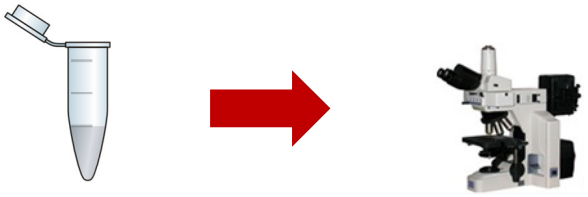


10 µl of semen
+ 1 µl of BLUE

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

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FluoVit

kit for sperm vitality assessment

FluoVit Protocol (concentration + motility + vitality)

CONCENTRATION, MOTILITY AND VITALITY ASSESSMENT UNDER FLUORESCENCE FOR HUMAN SPERM.

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

FluoVit is a very useful fluorescent staining solution that permits to calculate concentration, motility and vitality (RED=dead, BLUE=alive) at the same time during one analysis. This kit is composed by two fluorescent dyes and may be used with fluorescence microscopy.

The **FluoVit** kit is for the evaluation of sperm motility, concentration and vitality in fresh or unthawed semen samples. The kit is intended for standard semen analysis in routine assessment or research studies of male fertility.

200 µl BLUE + 200 µl RED = 200 analyses

Main characteristics of FluoVit

Storing conditions: Solutions should be stored at 2-6° degrees protected from light.

Caution: The stain solution is mutagen and should 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.

Fluorescent spectral characteristics: The **FluoVit** includes Trihydrochloride Trihydrate (330/380) and Propidium Iodide (536/617) based solutions stabilized for long lasting.

The microscope filter must be DAPI filter (EX 330-380, DM 400, BA 420, standard filter for UV). Recommended Xenon fluorescence light.

High resolution: High resolution images with higher contrast.

Mode of use

Step 1: Prepare 3 different empty vials **A**, **B and C** for the analysis.

Step 2: In the **vial A** put 250 µl of PBS (room temperature) with 1 µl of **BLUE vial** (at 37ºC, we recommend to heat only the numbers of microliters that are going to be used for the analysis of the day in a different vial in order not to damage the rest of the fluorochrome with temperature changes), and leave the **eppendorf A** in the incubator at 37ºC for 5 minutes.

In the same time, put 50 µl of **PBS** (room temperature) in the **vial B** and leave the eppendorf **B** in the incubator at 37ºC for 5 minutes.

Step 3: Add 100 µl of semen sample (fresh or unthawed) to the **eppendorf B**.

Step 4: Add 50 µl of **vial A** solution to the **vial B**.

Step 5: Leave the **vial B** in the incubator at 37ºC for 5 minutes minimum (or until BLUE sperm could be detected correctly).

Step 6: In the **vial C** put 5µl of vial **B** solution and add 1 µl of **RED** vial solution. Mix gently with the pipette. Pick up the mixed solution and fill in a counting chamber. Analyze motility, concentration and vitality in the same time under fluorescence using DAPI filter (you should see the sperms in two colors, red and blue) using the SCA® Program.

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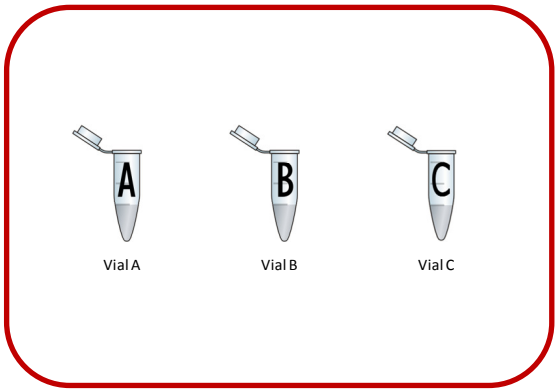

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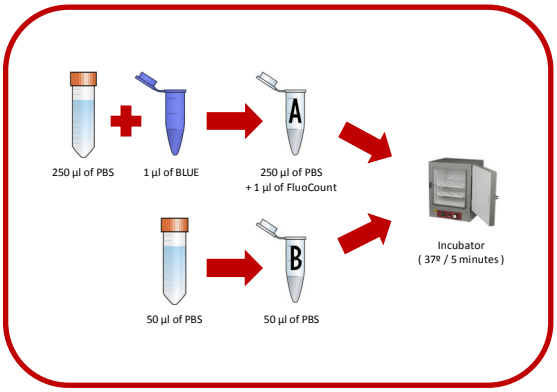

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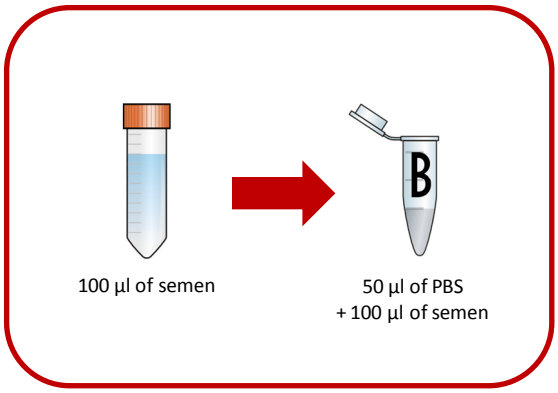

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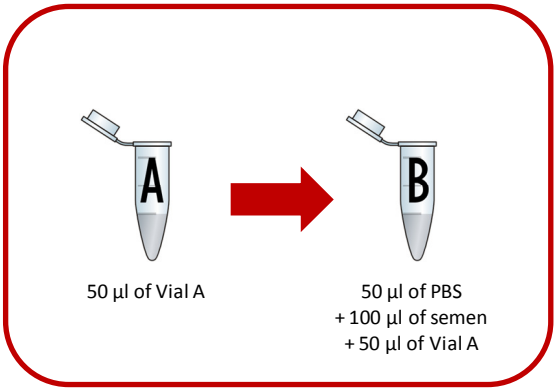

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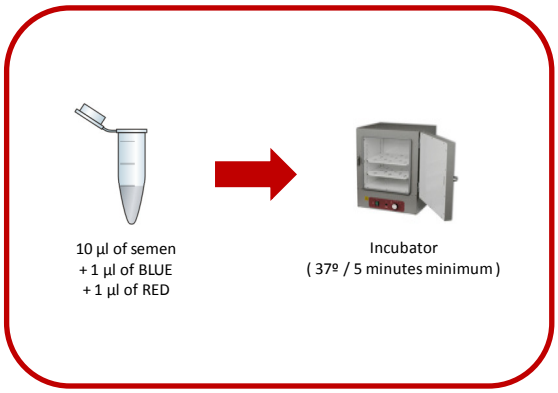

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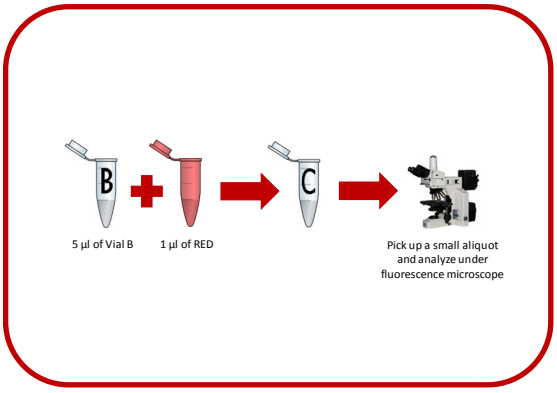

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