

ENHANCE-S Plus

ENHANCE-S Plus is a gradient system for semen preparation which consists of silane-coated colloidal silica particles suspended in HEPES-buffered EBSS (Earle's Balanced Salt Solution).

ENHANCE-S Plus may be stored at room temperature until opened.

ENHANCE-S Plus components

ENHANCE-S Plus Lower Layer (90%)

ENHANCE-S Plus Upper Layer (45%)

Additional materials needed

- 3cc syringes with 1 1/2" 21g needle
- Centrifuge (must be able to operate for up to 30 minutes at 350g-450g)
- Incubator or water bath at 37°C (optional).

Calculation of g-forces

The g-force of your centrifuge can be calculated using this formula:

$$g = 1.18 \times 100 \times \text{rpm}^2 \quad \text{or} \quad \text{rpm} = \text{Square root } \langle g / (1.18 \times r) \rangle$$

where:

r = radius of centrifuge in mm

rpm = rotations per minute / 1000

Example #1: r = 100mm, rpm = 1.5 (1500 rotations per minute)

$$g = 1.18 \times 100 \times 2.25 = 266$$

Example #2:

r = 100mm, g = 350 g

$$\text{rpm} = \text{SQR } \langle 350 / (1.18 \times 100) \rangle = 1.72 = 1720 \text{ rotations per minute}$$

Instructions for use with Fresh Semen Samples

1. Bring all components of the system and samples to room temperature or to 37°C.
2. Transfer 2.5 mL of ENHANCE-S Plus upper layer (45%) into a sterile disposable centrifuge tube.
3. Using a 3cc syringe with a 1 1/2" 21g needle, place 2.5 mL of ENHANCE-S Plus lower layer (90%) under the upper layer. Take care that the two layers are distinctly separated. This is done by placing the tip of the needle on the bottom of the test tube and slowly dispensing the ENHANCE-S Plus lower layer. This two layer gradient is stable for up to two hours.
4. Gently place up to 2.5 mL of liquefied semen onto the upper layer with a transfer pipette or syringe.
5. Centrifuge for 20 minutes at 350 g to 450 g. After centrifugation no pellet may be visible, however it is essential to continue the procedure with a second centrifugation.
6. Remove the supernatant down to the pellet.
7. Using a syringe, add 2-3 mL of sperm washing medium and re-suspend the pellet.
8. Centrifuge for 8 to 10 minutes at 300 g to 400 g. Higher sperm concentrations will require the maximum 10 minutes centrifugation to ensure a complete and thorough sperm wash.
9. Remove supernatant down to the pellet and repeat steps 7 and 8.
10. Remove supernatant and replace with a suitable volume of appropriate medium.

Instructions for use with frozen semen samples

1. Bring all components of the system and samples to room temperature or to 37°C.
2. Transfer 1.0 mL of ENHANCE-S Plus upper layer (45%) into a sterile disposable centrifuge tube.
3. Using a 3cc syringe with a 1 1/2" 21g needle, place 1.0 mL of ENHANCE-S Plus lower layer (90%) under the upper layer. Take care that the two layers are distinctly separated. This is done by placing the tip of the needle on the bottom of the test tube and slowly dispensing the ENHANCE-S Plus lower layer. This two layer gradient is stable for up to two hours.
4. Gently place 0.5mL of the thawed semen sample onto the upper layer using a pipette or syringe.
5. Centrifuge for 20 minutes at 350 g to 450 g.
6. Remove the supernatant down to no less than the 0.5 mL mark above the pellet.
7. Using a syringe, add 2-3 mL of sperm washing medium and re-suspend the pellet.
8. Centrifuge for 8 to 10 minutes at 300 g to 400 g.
9. Remove supernatant down to the pellet and repeat steps 7 and 8.
10. Remove supernatant and replace with a suitable volume of appropriate medium.

If samples do not liquefy and therefore do not pass through the layers, increasing the centrifugal force up to, but no more than, 600 g will help to separate the sperm.

STORE OPENED REAGENTS AT 2-8°C.



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