ENHANCE - S Plus with HTF Cell Isolation Media

For laboratory use only; other uses must be qualified by the end user

INTENDED USE

ENHANCE-S Plus with HTF is a gradient system for semen preparation, which consists of silane-coated colloidal silica particles suspended in HEPES-buffered HTF (Human Tubal Fluid, Modified).
Store ENHANCE-S Plus with HTF reagents at 2-8°C when not in use. Open and close bottles under aseptic conditions.
ENHANCE-S Plus with HTF reagents can be used in combination with IUI, IVF, and ICSI

ENHANCE-S PLUS COMPONENTS

ENHANCE-S Plus with HTF Upper Layer
ENHANCE-S Plus with HTF Lower Layer

REFERENCE NUMBER:

15254 ESPH-12
15252 ESPH-100-L
15250 ESPH-100

ADDITIONAL MATERIALS NEEDED

- 3cc syringes with 1 1/2" 21g needle
- Sterile, disposable conical centrifuge tube
- Centrifuge (must be able to operate for up to 30 minutes at 350g - 450g)
- Incubator or water bath at 37°C (optional).
- Enhance Sperm Wash medium or other sperm-washing medium

CALCULATION OF G-FORCES

The appropriate rpm for your centrifuge can be calculated using this formula:

$$rpm = \sqrt{[\, g / (1.118 \times r) \,]} \quad \text{or} \quad g = 1.118 \times r \times rpm^2$$

where: r = radius of centrifuge in mm rpm = rotations per minute / 1000

Example #1: To Achieve 350g, If r = 100mm, g=350

$$rpm = \sqrt{[\, 350 / (1.118 \times 100) \,]} = 1.77 = 1770 \text{ rotations per minute}$$

Example #2: If r = 100mm, rpm = 1.8 (1800 rotations per minute)
g = 1.118 x 100 x 3.24 = 362 g

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 with HTF upper layer 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 with HTF lower layer 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 with HTF 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 15 - 18 minutes at 350g to 400g. When this centrifugation is complete you may not be able to visibly see a pellet. If there is no pellet continue centrifugation for an additional 3 minutes. Do not use the brake to stop the centrifuge.
6. Remove the supernatant down to the pellet.
7. Using a syringe, add 2-3 mL of Enhance Sperm Wash Medium or appropriate medium and re-suspend the pellet.
8. Centrifuge for 8 to 10 minutes at 300g. 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. (Repeat steps 7 and 8 if an additional wash is desired.)
10. Remove supernatant and replace with a suitable volume of Enhance Sperm Wash Medium or 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 with HTF upper layer 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 with HTF lower layer 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 with HTF 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 350g.
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 Enhance Sperm Wash Medium or appropriate medium and re-suspend the pellet.
8. Centrifuge for 8 to 10 minutes at 300g.
9. Remove supernatant down to the pellet. (Repeat steps 7 and 8 if an additional wash is desired.)
10. Remove supernatant and replace with a suitable volume of Enhance Sperm Wash Medium or appropriate medium.

If samples do not liquefy and do not pass through the layers, increase the centrifugal force up to, but no more than, 500g to help separate the sperm. Modification of this procedure may be necessary to optimize semen preparation results.

PRECAUTIONS AND WARNINGS

CAUTION: Federal Law restricts this device to sale by or on the order of a physician (or properly licensed practitioner). For external use only; not for injection. Do not use any bottle of medium showing evidence of particulate matter or contamination. To avoid problems with contamination, practice sterile transfer technique and discard minimal amounts of excess medium remaining in the bottle.

STORAGE INSTRUCTIONS AND STABILITY

Store products between 2-25 °C. Once opened:

- Store between 2-8 °C.
- Store product between 2-8 °C.
- The products can be used up to 7 days after opening when sterile conditions are maintained, and the products are stored at 2-8 °C.
- Keep away from (sun) light.
- The products are stable after transport (max. 5 days) at elevated temperatures (≤ 37 °C).
- The devices need to be disposed in accordance with local regulations for disposal of medical devices.

PRODUCT SPECIFICATIONS

A complete Certificate of Analysis is provided with every lot of Enhance-S Plus Cell Isolation Medium.

RELATED PRODUCTS

Vitrolife has a full line of products for the Reproductive Medical Specialist. For more information or technical support please visit our website at www.vitrolife.com or contact Customer Service at (866) 848-7687. Email contact and information at order.us@vitrolife.com.