

Mouse IVF Kit Protocol

(updated 10/22/2020)

A. Kit components: 1 kit is enough for two IVF procedures

	1 Kit	½ Kit
RVF+Ca or mHTF medium	4 x 10 ml in 15ml tubes	2 x 10 ml in 15ml tubes
MBCD medium	2 x ≥1 ml in 5ml tubes	2 x ≥1 ml in 5ml tube
GSH (L-glutathione reduced)	22.5 mg (0.0225 g) in 5ml tube	22.5 mg (0.0225 g) in 5ml tube

B. Kit preparation and storage before shipping:

1. Prepare liquid components inside a laminar flow hood. Check and make sure each component is included with tube intact, closed and sealed tightly.
2. Place the tubes in a strong plastic bag and seal the bag. If shipping in a LN2 dry shipper, organize or orientate the tubes so that the kit can be placed into a dry shipper canister easily.
3. Kit storage temperature before shipping:
 - If shipping in a cold box: 4 °C.
 - If shipping in dry ice or liquid nitrogen dry shipper: - 80 °C.

C. Shipping methods:

- **If shipping in a cold box:** Place the kit into a Styrofoam box filled with frozen ice packs. Upon arrival at the customer's lab, stand each tube on a rack, store at 4° C, and use within 2 weeks.
- **If shipping in dry ice:** Place the kit in a Styrofoam box filled with dry ice. Upon arrival at the customer's lab, store the kit at -20 or -80° C (-80 °C better), and use within 2 months. After the kit is thawed, store the media at 4 °C, and use within 2 weeks.
- **If shipping in a liquid nitrogen dry shipper:** Place the kit into the canister of a precooled dry shipper. Upon arrival at the customer's lab, remove the kit from dry shipper, and store at -80 or -20° C (-80 °C better), and use within 2 months. After the kit is thawed, store the media at 4 °C, and use within 2 weeks.

D. How to use the kit:

1. RVF or mHTF medium with high Ca concentration: See IVF protocol. Frozen-thawed medium must be gently inverted a few times to mix prior to use.
2. MBCD medium: See IVF protocol. Frozen-thawed medium must be gently inverted a few times to mix prior to use.
3. GSH stock solution (100 mM): On the morning of IVF, add 0.75 ml RVF+Ca or mHTF medium to the 22.5 mg GSH powder to dissolve, and then filter sterilize using a 0.2 µm syringe filter. Remaining GSH solution can be aliquoted and stored at -80 or -20 °C for later use. See IVF protocol (not included in IVF kit) for use of 100 mM GSH solution.