Tissue Transplantation Protocol

Solutions

25x Ringers (Ca2+ free) 500ml

NaCl 84.75 g (2.9M)
KCl 2.7 g (0.07M)
H2O to 500ml
Autoclave
HEPES 14.9 g (.12M)

100x Ca2+ solution 500ml

CaCl 2+2 H2O 13.2 g (0.1M)
Autoclave

1x Ringers

25x Ringers 40ml
100x Ca2+ 10ml
H2O to 1000ml
Adjust to pH 7.2 with 1N NaOH (about 0.5-1.0 ml)

1/3x Ringers

25x Ringers 10ml
100x Ca2+ 7.5ml
H2O to 750ml
Adjust to pH 7.2 with 1N NaOH (about 0.5-1.0 ml)

Cleared chicken egg whites

Whip egg white from one chicken egg with a whisk until the whites turn glossy and their tips stand tall. Leave for 20 minutes, and collect clear egg white solution from beneath foam. Should get about 10ml.

1x Ringers/1.6% Egg white

Egg white 1.6ml
1x Ringers 100ml

Pronase solution

500ul 30% pronase
25ml E3 (or egg water)

Other equipment
Glass or agarose petri dishes for dechorionation and recovery
Agarose bed petri dish for surgery
Hair knife and hair loop
Or
Capillary knife and capillary probe. Using TW100F-4 capillary (normally used for injections) pull using program normally used for transplant needles. This provides two long needles with elongated tapers. Fire one of the two needles points to create a polished probe for handling embryos. The taper of the other needle is used as the knife. A bend fired into the shaft of the needle may help in handling the knife. For easy handling of the probe and knife, insert the open capillary ends onto 23 gauge hypodermic needles, glue into place, and place onto syring. This allows you to hold the probe and knife like a pencil.

Transplant
1) Collect embryos (host yolk strain and donor blastoderm strain) and dechorionate with pronase solution or manually in a glass petri dish or agarose bed.

2) Perform blastoderm transplants at desired stage (around 512 1000 cells). Transfer two strains of embryos to petri dish with agar bed in 1 x Ringers/1.6% egg white. Addition of egg whites helps in the quick healing of transplant.

3) Using either a hair knife or a pulled glass capillary knife and a polished glass capillary probe remove blastoderm from the host yolk. Remove blastoderm from blastoderm host. It is best to prepare the yolk first as the donor blastoderm will begin to ball up shortly after it is prepared.

4) Position the cut face of the donor blastoderm onto the cut surface of the host yolk where the original blastoderm was positioned. Gently put pressure on the yolk and blastoderm for a few seconds to allow the two surfaces to adhere to one another.

5) Allow 5-10min healing time in Ringers/Egg whites then transfer embryos to glass petri dish or agarose bed containing 1/3x Ringers using a glass transfer pipet. The high salt concentration of 1x Ringers prevents further development, so they must be transferred out.