# CRE Treatment of Embryos

## 1.0 Equipment

**1.1** Embryo Handling Device

**1.2** Incubator (37°c, 5% $CO_2$)

**1.3** Powerpette

**1.4** Gilson Pipettes (P20, P200)

**1.5** Vessel for ice

**1.6** Countdown timer

**1.7** Microscope

**1.8** Heated Stage (37°C)

**1.9** Safety glasses

**1.10** Cyclone Vortex Mixer

**1.11** Falcon tube rack

## 2.0 Supplies

**2.1** Silicone Fluid

**2.2** 10ml serological pipettes

**2.3** Human tubal fluid (hTF)

**2.4** Falcon Tube

**2.5** DMEM

**2.6** Scientific

**2.7** 0.5ul aliquot of CRE Recombinase Enzyme

**2.8** 35:3004 Culture Dishes

**2.9** Gilson Pipette Tips (20µL, 200µL)

**2.10** Wedged Pipette Tips

**2.11** Tips for Embryo Handling Device

**2.12** Ice

**2.13** M2 Medium

**2.14** Permenant Marker Pen

## 3.0 General Information

**3.1** The CRE Recombinase enzyme is aliquoted (into 5µL aliquots), tested and stored at -80°C.

**3.2** The aliquots **MUST** be transported to the lab on wet ice.

**3.3** The enzyme should be removed from the freezer only when it's ready to be used. Ensure the dish equilibration time and the CRE treatment time are adhered to.

**3.4** Depending on the concentration of the stock CRE enzyme and the concentration required of the final solution, varying amounts of DMEM and CRE enzyme are required. This has been calculated and placed into dilution tables (See Appendix 1). The concentration required of the final solution is determined during the CRE test.

**3.5** If frozen embryos are being treated with CRE, thaw according to appropriate protocol then wash in 2 drops of M2 prior to treatment.

**3.6** The embryos are CRE treated at the 2 cell stage on the day of embryo transfer.

**3.7** Once the IVF have been scored, the dishes for the CRE treatment can be prepared. If there is an excess of embryos, only treat enough for the transfers plus a few extra. After that, any excess embryos could be frozen, without the allele conversion.

## 4.0 Preparing dishes for CRE treatment:

**4.1** Label the embryo culture dish with the IVF No. and stock (one dish is required for each IVF undergoing CRE treatment). A spare dish should also be prepared (labelled 'spare'). On the underside of the dish '1' should be written on the top to help orientate the dish.

**4.2** Add approx. 3-4ml of DMEM into a falcon tube. The DMEM is stored in the fridge and can be used for 3 months after opening. If out of date, discard and use a fresh bottle.

**4.3** The required amount of DMEM can be determined using the dilution tables in Appendix 1.

For example; if the stock enzyme was at a concentration of 15mg/mL and the required concentration determined by the CRE test is 1.2µM, then 580µl of DMEM is required along with 2µL of enzyme.

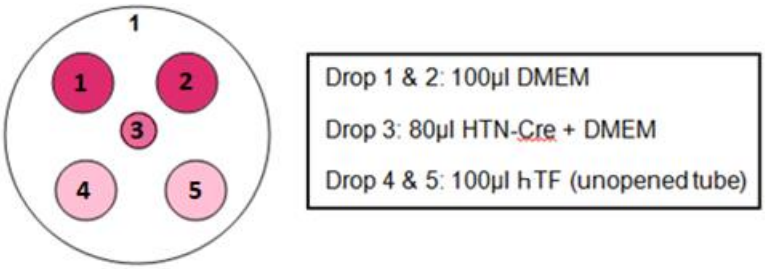
**4.4** Add the required amount of DMEM to an Eppendorf tube. At this stage, the CRE enzyme can be collected from the freezer on ice. The required amount of CRE can be added to the Eppendorf of DMEM.

**4.5** The solution should be mixed by vortex for 5 seconds.

**4.6** The dishes should be prepared as follows (Picture 1):

- Drops highlighted as 1 and 2 in Picture 2 are 100µL of DMEM (use leftover DMEM in the falcon tube).
- Drop 3 is 80µL of DMEM + CRE
- Drops 4 and 5 are 100µL of hTF. Ensure a fresh tube is used.
- Overlay the dish with silicone fluid.

**Picture 1**




**4.7** Place the dishes in the incubator to equilibrate and start a timer for 20 mins. Make a note of the time the dishes went into the incubator.

## 5.0 CRE treatment of the embryos

**5.1** After 20mins has elapsed, remove the CRE dish from the incubator, along with the embryos required for treatment.

**5.2** Using an embryo handling device, place the embryos into drop 1 in the dish, pre-loading the tip from drop 1. Change the tip then move the embryos to drop 2, pre-loading the tip from drop 2.

**5.3** The embryos should be picked up and replaced into different areas within drop 2, 3-4 times.

**5.4** The tip should be changed again and the embryos should be placed in drop 3, pre-loading the tip from drop 3.

**5.5** Place the dish back into the incubator and start a timer for 40 mins. Make a note of the time the embryos were placed into drop 3.

**5.6** Once the treatment time has elapsed, use a 20µL Gilson pipette to remove 20µL of hTF from drop 4 and expel it into drop 3 to stop the action of the enzyme.

**5.7** Use an embryo handling device to wash the embryos in drop 4 and 5, changing the pipette tip between drops. The embryos can be kept in drop 5 and placed back into the incubator until ready to transfer. Make a note of the time the embryos were washed.

## 6.0 Appendix 1

| 15mg/ml Cre Dilution Table | | |
|---|---|---|
| Required Concentration | Cre Vol. | DMEM Vol. |
| 0.3µM | 1µl | 1936µl |
| 0.6µM | 1µl | 581µl |
| 1.2µM | 2µl | 580µl |
| 1.8µM | 3µl | 579µl |
| 2.4µM | 4µl | 576µl |
| 3µM | 5µl | 575µl |

| 10mg/ml Cre Dilution Table | | |
|---|---|---|
| Required Concentration | Cre Vol. | DMEM Vol. |
| 0.3µM | 1µl | 774µl |
| 0.6µM | 2µl | 773µl |
| 1.2µM | 4µl | 772µl |
| 1.8µM | 4µl | 512µl |
| 2.4µM | 4µl | 384µl |
| 3µM | 4µl | 306µl |

| 6mg/ml Cre Dilution Table | | |
|---|---|---|
| Required Concentration | Cre Vol. | DMEM Vol. |
| 0.3µM | 1µl | 463µl |
| 0.6µM | 2µl | 463µl |
| 1.2µM | 4µl | 464µl |
| 1.8µM | 4µl | 309µl |
| 2.4µM | 4µl | 232µl |

| 4.5mg/ml Cre Dilution Table | | |
|---|---|---|
| Required Concentration | Cre Vol. | DMEM Vol. |
| 0.3µM | 3µl | 1044µl |
| 0.6µM | 3µl | 520µl |
| 1.2µM | 3µl | 259µl |
| 1.8µM | 6µl | 343µl |
| 2.4µM | 6µl | 256µl |
| 3µM | 6µl | 203µl |

| 2mg/ml Cre Dilution Table | | |
|---|---|---|
| Required Concentration | Cre Vol. | DMEM Vol. |
| 0.3µM | 3µl | 462µl |
| 0.6µM | 6µl | 459µl |
| 1.2µM | 9µl | 340µl |
| 1.8µM | 12µl | 298µl |
| 2.4µM | 12µl | 221µl |
| 3µM | 12µl | 174µl |