



## MEDIA :

M16 (Sigma M7292)

Sucrose 1M : M2 + sucrose (342mg/ml)

Mineral Oil (Sigma réf : M8410)

## INSTRUCTIONS

Remove straw from liquid nitrogen storage and hold horizontally in room air until the ice disappears

Wipe the straw and cut away the two ends in order to expel immediately the contents into a slide (Dutscher, Cat#020302). (If such a slide is not available, a Petri dish can be used.)

Under microscope recover the embryos in minimal amount of media and transfer into a drop of 40 $\mu$ l of Sucrose (1M).

For each straw to be thawed prepare a 50 $\mu$ l microdrop of M16 in 4 wells plates. Allow media to equilibrate in incubator (5%Co<sub>2</sub>, 37°C) for at least 15 minutes prior to use.

When the embryos start to shrink add some microdrops (approximately 10 $\mu$ l) of M2. Recover the embryos from Sucrose + M2 and wash through five 40 $\mu$ l-drops of M2 into a 40 $\mu$ l-drop of M16.

Select the embryos with normal appearance and transfer them into the equilibrated drop of M16. Add mineral oil in the wells and return the embryos to incubator for two hours.

Embryos at 2-cells or 4-8 cells stage can be transferred into the oviducts of 0.5 day pseudopregnant recipients. The morula or blastocysts should be transferred into the uterus of 2.5 day pseudopregnant females.

