Orders: orders@novusbio.com
Support: technical@novusbio.com
Web: www.novusbio.com

Protocols, Publications, Related Products, Reviews and more:
www.novusbio.com/NBP2-25171

NBP2-25171 Protocol

Serum protocol for DGCR8 knockout MEF cells (NBP2-25171)

DGCR8 knockout MEF cells: https://www.novusbio.com/products/dgcr8-knockout-mef-cells_nbp2-25171
This protocol is written for growing cells in T25 tissue culture flasks, please make changes accordingly for flasks of different sizes

Required Medias:

MEF for embryonic fibroblasts:
DMEM-Hi glucose 425 ml (Caisson Labs, DML10-500ML)
FBS 75 ml (Denville Scientific, FB5001)
100 X non-essential amino acid 5 ml (Millipore EmbryoMax(R) TMS-001-C)
200 mM L-Glutamine 5 ml - (Sigma G7513)

Protocol:

1. Bring up MEF cells in a T25 flask:
a. Put 10 mls of MEF media into 15ml conical vial.
b. Warm vial of cells for 1-2 minutes in 37 degree water bath.
c. Gently add thawed cells to MEF media in conical vial.
d. Spin down for 5 minutes @ 1000 RPM to obtain cell pellet.
e. Aspirate freeze media and resuspend pellet in 8 mls of fresh MEF media, transfer full amount to a T25 flask
f. Rinse and feed the following day to remove aggregates

2. Transferring MEF cells to a T75 flask:
a. Once cells are confluent (should only take 2 days), rinse 1X with 2 mls of sterile 1XPBS
b. Add 2mls of Trypsin and incubate ate 37 degrees Celsius for 2 minutes
c. Cut trypsin with 2mls of MEF media and transfer full amount into T75.

Freezing down DGCR8 MEF KO's:
1. Trypsinize flask of desired volume
2. Collect entire cell split and spin down to obtain cell pellet
3. Resuspend pellet in freeze media (MEF media + 10% fresh FBS + 10% DMSO, sterile filtered) at desired concentration
4. Transfer into cryogenic labeled vials
5. Put overnight in -80 degree freezer
6. Transfer to liquid nitrogen for long term storage