

MNT-1 Cells

MNT-1 Medium

(Sterile filter as making)

		<i>Final</i>
450mL	DMEM	
100mL	FBS*	20%
5mL	Pen/Str	1x
5mL	L-glut	1x
50mL	AIM-V	10%
5mL	NEAA	1x
5mL	Na Pyruvate	1x

*The new lot has not been tested; use older lots.

To Carry

Split 1:10 if you do not need them for awhile (> week)

1:5 if you need them in 3-5 days

1:2 if you need them the next day

*Do not split back more than 1:10 as it takes forever to recover

Freezing

Freezing medium: 75% DMEM
15% FBS
10% DMSO

Grow cells until they are almost, but not actually, confluent. You want an active growth phase. If they are stationary when you freeze them, they may not recover.

For one T75 flask (normally try to freeze 2-3 T75 flasks)

1. Trypsinize the flask
2. Add 10 mL media to kill trypsin activity
3. Pellet cells 5' 800rpm table top
4. Aspirate media
5. Add 1.5mL freezing media

6. Resuspend by pipetting
7. Aliquot 500 μ L/tube in cryovials labeled with cell line name, passage #, date, your initials. Put in styrofoam container in -80 C overnight. The next day. transfer to liquid nitrogen tank without letting them thaw.

****Record entry in log book****

Thawing

Add 15mL **cold** medium to a T75 flask. Go to the liquid nitrogen tank and remove tube, placing it in 37 C to thaw it quickly. When it has **just** thawed, add cells to the flask.

Ethanol the tube well.

****Record removal in log book****

Next day, check culture under the microscope. I normally change the media at this point to get rid of dead floating cells and DMSO. However, if a lot of live floaters (have halo around them) are present, I do not change the media and instead give them 1-2 more days to sit down.

Transfection

(Notebook 3-179)

-Lipofectamine \rightarrow need to titrate for IF (for western: 2 μ g DNA/8 μ g lipofectamine)
-cells do not sit down well on coverslips

- Day 1 \rightarrow plate cells (probably 1:25 from T75)
- Day 2 \rightarrow transfect
- Day 3 \rightarrow split
- Day 4 \rightarrow
- Day 5 \rightarrow fix

(Matrigel to get sitting down?)

Maybe try Fugene again (1 time tried and it didn't work)