

## General Protocol: Freezing Cells

### To Freeze Cells:

- ❖ Add 2 mls of freezing solution (Recovery Cell Culture Freezing Media, Cat #12648-010) and triturate to loosen the pellet.
- ❖ Split the 2 mls of freezing solution into 3 equal quantities and place in 3 separate cryovials
- ❖ If the cells are grown on a P10 and not too confluent, use 1 ml and transfer the entire 1 ml to a 2 ml cryotube to freeze. If the cells are grown on a T75 and are quite confluent, use 2 mls of freezing media and transfer 1 ml to each of 2 cryotubes.
- ❖ Place the cryovials in a -20°C freezer for a quick freeze for ~1 hour, preferably in a cell freezer box stored at -20°C.
- ❖ Remove from the -20°C freezer and immediately place in a -80°C freezer overnight (or up to 1 week).
- ❖ Transfer cells from -80°C freezer into a container with dry ice for transfer into liquid nitrogen.
- ❖ Place cells in liquid nitrogen.
- ❖ Note: Any thawing in the freezing media may result in cell death when trying to thaw the cells in the future