THAWING CELL LINES

Changes to the protocol in red.

*Read **ALL** information below before you start thawing*

THAW CELLS. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the cap out of the water. Thawing should be rapid (approximately 2 minutes). Remove the vial from the water bath as soon as you only see a small clump of ice (do not wait until the complete contents are thawed), and decontaminate by spraying with 70% ethanol.

REMOVE DMSO. Gently transfer the vial contents (using a disposable transfer pipette) to a 15 ml centrifuge tube containing 10 ml warm complete culture medium (to dilute the DMSO) and spin at 130 x g for 5 minutes at room temperature.

START CULTURE. Resuspend the cell pellet with the appropriate cell line medium (amount depends on the number of living cells in the cryo vial) and dispense into a 25 cm2 culture flask.

293T cells (adherent):	60,000 cells/cm^2 will result in 40-50% confluence after 1 day.
	Regular seeding: 15,000 / cm^2 (2 days) or 6,000 / cm^2 (3 days)
3T3 cells (adherent):	Don't know; didn't buy them from ATCC
	Regular seeding: 4,000 / cm^2 (3,5 days)
THP1 cells (suspension):	200,000 / ml → 250,000 – 350,000 / ml after 5 days
	Regular seeding: 150,000 – 200,000 / ml (2 days) or 80,000 – 100,000 / ml (3 days)

NOTE. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that the 25 cm2 culture flask containing the medium is placed in the incubator for at least 15 minutes prior to the addition of the cells to allow the medium to reach its normal pH (7.0 to 7.6).