innovative cell technologies

Typical Stem Cell Passaging Protocol Using Accutase® GMP

AccutaseGMP is formulated at a concentration that is ready to use, once defrosted. (**Note: Never defrost a bottle of AccutaseGMP at 37°C**.) A defrosted bottle of AccutaseGMP can be removed from the refrigerator and immediately applied to cells. It does not need to be and should not be pre-warmed to 37˚C. AccutaseGMP contains proteolytic and collagenolytic enzymes to gently break down the cell adhesion structure on the outside of cells that attaches them to the bottom of the flask.

This entire procedure should be done in a laminar flow hood using proper aseptic technique.

1. Carefully aspirate all of the media from the cell culture flask. (Rinsing with PBS is not necessary.)
2. Immediately add enough 4°C AccutaseGMP to the flask to cover the cells. (Typically, 0.1 to 0.2 mL per cm^2 of culture vessel surface area depending upon confluency and density of the cell culture.)

Flask	Approx. Growth Area (cm^2)	Recommended AccutaseGMP Volume (mL)
T25	25	2.5 – 5
T75	75	7.5 – 15
T150	150	15 – 30
T175	175	17.5 – 35
T225	225	22.5 – 45

3. Place the flask in a 37°C incubator for 5 to 10 minutes up to a maximum of 1 hr. After 5 minutes, check the flask every 2-3 minutes for cell detachment.
4. Once ~90% of the cells have detached, smack the flask against the palm of your hand to dislodge any remaining cells.
5. Add an equal volume of culture media to the flask, rinsing the culture surface with the media.
6. Gently resuspend the cell suspension and take a sample to determine the viable cell density.
7. Add the desired volume of cell suspension based on target culture density to fresh media in new flasks. Evenly distribute the suspension to ensure even seeding and place the flasks into the 37°C incubator. No neutralization steps are required. Cells will reattach within an hour depending upon cell type.

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