



Typical Suspension 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.

The purpose of this procedure is to disassociate aggregated stem cells cultured in a suspension vessel into a single cell suspension for passaging. This entire procedure should be done in a laminar flow hood using proper aseptic technique.

1. Allow cells to settle to the bottom of the culture vessel. Depending on cell aggregate size centrifugation may be necessary.
2. Carefully aspirate all of the media from the cell culture vessel. (Rinsing with PBS is not necessary.)
3. Immediately replace the minimum culture volume with 4°C AccutaseGMP in the vessel.
4. Resume culture agitation and place the vessel in a 37°C incubator for 5 to 10 minutes up to a maximum of 1 hr. After 5 minutes, check the vessel every 2-3 minutes for cluster disbursement.
5. Once ~90% of the aggregates have been disassociated into a single cell suspension return the vessel to the laminar flow hood.
6. Add an equal volume of culture media to the vessel, pipetting up and down to gently break apart any remaining aggregates.
7. Gently resuspend the cells and take a sample to determine the viable cell density.
8. Add the desired volume of cell suspension based on target culture density to fresh media in new vessels. Return the vessels into the 37°C incubator. No neutralization steps are required. Cells will reaggregate within an hour depending upon cell type.

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