Celase® GMP

Worthington Biochemical Corporation

Protocol

Isolation of Progenitor and Stem Cells from Rabbit Adipose Tissue

⚠ All procedures must be performed in biosafety hoods.
⚠ This procedure is intended for processing rabbit subcutaneous adipose tissue only.
⚠ Surgically remove the inguinal fat pad using sterile techniques.
⚠ Using fresh adipose tissue will yield the best results.

❶ Pre-warm the processing solution (sterile PBS or sterile Lactated Ringer's) to 37˚C. Measure the tissue weight in a pre-zeroed sterile container; this will be used to calculate the processing solution volume and Celase® GMP volume.

❷ Wash the adipose tissue with processing solution if bloody.

❸ Use surgical scissors to mince the tissue to a 3-5 mm^3 size or until no obvious large pieces observed. Add a small amount of processing solution if the tissue is too dry.

❹ Reconstitute the Celase GMP vial with 5 mL of the processing solution. Invert the vial several times to fully reconstitute the enzyme into a clear solution (reference Celase GMP IFU).

❺ Place the washed and minced tissue into a sterile container, usually 6 times the size of the original tissue volume. For a given amount of tissue, add the appropriate volumes of processing solution and Celase GMP to the container; tightly close the container.

Adipose Tissue	Processing Solution	Celase GMP
5 mL	15 mL	0.5 mL
10 mL	30 mL	1.1 mL
15 mL	45 mL	1.6 mL
20 mL	60 mL	2.1 mL
25 mL	75 mL	2.6 mL
30 mL	90 mL	3.2 mL
35 mL	105 mL	3.7 mL
40 mL	120 mL	4.2 mL
45 mL	135 mL	4.8 mL
50 mL	150 mL	5.3 mL

❻ Place the container in a 37˚C shaking water bath for 60 ± 15 minutes until the tissue becomes visibly broken down. If a shaking water bath is not available, gently swirl the container contents by hand continuously or in 5 minute increments for 60 ± 15 minutes.

❼ Collect the non-buoyant solution into multiple 50 mL centrifuge tubes. Place the tubes in a centrifuge and concentrate the cells at 600 g for 5 minutes at room temperature with a low-medium brake speed.

❽ Carefully aspirate the top lipid, floating layer and the solution that contains Celase GMP.
⚠ Do not disturb the cell pellet and leave 5-10 mL of the solution in the tube.

❾ Resuspend the cell pellet in each 50 mL tube with the remaining solution. Combine the cell pellets from each tube and then wash with the processing solution 2 times to reduce residual enzyme. Resuspend the combined cell pellet with 25-30 mL of the processing solution and pass through a 100 µm strainer followed by a 40 µm strainer.

❿ Centrifuge 1 more time and resuspend with the remaining processing solution into the final desired volume for cell counting and further *in vitro* and *in vivo* studies.