ISOLATION OF HEPATOCYTES FROM MOUSE LIVER

Enzymes

COL G recombinant collagenase class I ACTIVITY ≥3.0 Units/mg (Pz Grassmann)

COL H recombinant collagenase class II ACTIVITY ≥30.0 Units/mg (Pz Grassmann)

Preparation of recombinant collagenases COL G and COL H, and Thermolysin solution:

1. Resuspend the lyophilized COL G in sterile H_2O and filter for sterility. Do not exceed 30 U/ml when resuspending. Store in aliquots containing at least 50 U_{total} each at -20 °C.

2. Resuspend the lyophilized COL H in sterile H_2O and filter for sterility. Do not exceed 300 U/ml when resuspending. Store in aliquots containing at least 300 U_{total} each at -20 °C.

3. Dissolve Thermolysin (from Geobacillus or other) in DMEM low-glucose at a concentration of [1 mg/ml] and filter for sterility; aliquot and store at - 20 °C.

4. For the extraction of a liver from one mouse (weight between 15 and 30 grams), prepare 100 ml of a solution in DMEM low glucose + 1% Antibiotics (Penicillin and streptavidin) + 15 mM HEPES. Immediately before use add 50 U COL G + 300 U COL H + Thermolysin [0.01mg/ml]. This is the DIGESTION SOLUTION.

5. Prepare 100 ml of a solution of PBS without Ca^{2+} and Mg^{2+}, containing 0.5 mM EGTA and 25 mM HEPES pH 7.4. This is the WASHING SOLUTION.

Hepatocytes isolation from mouse

1. Euthanize the mouse (weight between 15 and 30 gr) by cervical dislocation.

2. After disinfection by alcohol and iodine solution, transfer it into a laminar flow hood and position in supine view on an inclined plane support. Using curved scissors with pliers help practicing longitudinal incision at the medial plane of the abdomen, then cut through the skin from the pelvis to the sternum.

3. Make the reversal of abdominal peritoneum muscles, exposing the viscera in order to have an easy access to the portal vein (VP) so as to be able to insert a cannula and maintain perfusion. Insert the cannula and start the perfusion with the WASHING SOLUTION with a flow rate of 2 ml/min. When the liver "bleaches" (becomes clear), cut the swollen vena cava (VC) to reduce the pressure and increase the flow rate to 9 ml/min in continuous perfusion with the WASHING SOLUTION for 10 minutes.

4. Continue perfusion with 70 ml of DIGESTION SOLUTION at the same flow rate (9 ml/min).

5. Following the perfusion, withdraw the liver from the animal and place it into a 50 ml conical tube containing 10 ml of DIGESTION SOLUTION and incubate in a thermostatic bath at 37 ° C for 10', stirring (100 rpm).

6. Add 20 ml DMEM-low glucose + 1% Penn-Strep + 15 mM HEPES.

7. Use a 25 ml serological pipette to homogenate the digested tissue.

8. Filter through a gauze (100 μm mesh) to remove debris and undigested tissue, then centrifuge at 50*g* for 2' at 4 °C.

9. Wash the pellet three times in 30 ml of DMEM-low glucose + 1% Penn-Strep + 15 mM HEPES.

10. Remove the supernatant and resuspend the hepatocytes in 10 ml DMEM/F-12 + 1% Penn-Strep.

11. Count the hepatocytes and seed into a petri dish, previously coated with rat tail type-I collagen (50 μg/ml), about 3 x 10^5 cells/dish.

12. Incubate at 37 °C, 5% CO_2, 95% O_2 about 1h; then change the medium with DMEM-low glucose + 1% Penn-Strep + 10% FBS.

13. After 4-5 hours in culture (necessary time for hepatocytes adhesion) change the medium with the William's Media E to preserve the original morphology.

Note: *This protocol is meant to be a starting point; all isolation procedures require an individual optimization. COL G and COL H concentration, protease addition and digestion time can be experimentally adjusted.*

If you have any question please contact info@abielbiotech.com