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PROTOCOL TO HARVEST CELLS FROM BioNOC™ II CARRIERS

Contents

Introduction

One of the reason which makes our BioNOC II™ carriers advantage over other commercially available macrocarriers is its ability to allow wide variety of cell types to attach efficiently and grow on without the need to perform extra coating. To ensure efficient cell harvesting from BioNOC II carriers, a standard protocol is as below.

(Disclaimer: Due to the nature of each cell type, different enzymes may be needed for the proper detachment. Enzyme type, concentration and incubation time need to be optimized to obtain best cell harvest efficiency)

Materials

- Accumax,
- Trypsin 0.25%
- TrypLe Express
- Collagenase
- PBS pH 7.2

Method (General Protocol)

1) Rinse carriers with PBS (0.3ml-1ml per carrier); Suction

2) Repeat step 1 for 4 more times

Hint*: Carriers tend to retain media due to strong hydrophilicity property. If growth media contains FBS and are not fully removed from carriers, remaining FBS can inactivate certain enzymes such as trypsin

3) Add an appropriate amount of dissociating enzyme such as 0.25% Trypsin-EDTA to carriers, incubate for 10 min 37˚C; no shaking required

4) Perform enzyme dissociation:

i. Trypsin/ TrypLE Express: (most cell types)
   - Add 1 ml 0.25% Trypsin-EDTA, incubate at 37ºC for 10-15 min. Add in 1 ml of neutralization media.

ii. Accumax/ Accutase: (suitable for stem cells)
   - Add 1 ml Accumax/ Accutase, incubate at room temperature for 15 - 30 min. (Incubation time depends on cell density, we would suggest a study with 15 min, 20 min and 30 min). Accumax is recommended for cells growing in 3D. However, you may use your preferred dissociation agent/method.

iii. Collagenase: (suitable for stem cells)
   - Dilute collagenase type II (Thermo Scientific, Cat 17101) to achieve final working solution of collagenase containing 100 units/ml and 5 mM of $CaCl_2$ dissolved in PBS.
   - Add 1 ml of collagenase and incubate for 15 - 30 min. (Please optimize duration of collagenase as required).

6) Holding the cap/top of the tube with index and thumb, flick bottom of tube with solid rod (pen or metal ruler) to dislodge cells for 20-30s.

7) Harvest dislodge cell suspension into a clean tube

8) Repeat harvest with PBS (0.3-1ml per carrier) as step 7 and 8 for 4 more times.

9) Pool all harvest; spin at 1,500 rpm 3 min 4˚C

10) Count cell pellet on haemocytometer