

CULTURING HPDE CELLS

Medium:

1. Keratinocyte SFM, + EGF + bovine pituitary extract (Invitrogen Cat#:17005042) supplemented with 1x antibiotic-antimycotic (Gibco Cat#15240-062)

Passaging:

1. Wash the cells with 5ml Hanks or PBS once (100mm plate)
2. Add 1ml of 0.5% TrypsinEDTA (Invitrogen Cat#15400-54) and leave in the incubator for 5-10 minutes. Note: if cells do not detach easily add a bit more trypsin.
3. Neutralize trypsin by adding the same volume of 0.1% trypsin inhibitor (Invitrogen, Cat#17075-029), then add 2 ml of medium and spin, discard the supernatant and plate the cells on new plate (8-10ml of growth media).

Notes:

Splitting ratio should not exceed 1:4 and the medium should be replaced every 2-3 days.

On average these cells are split 1-2 times a week depending on your experiments.

HPDE cells should not be split too thinly and are very difficult to subclone from single cells.

Using trypsin inhibitor and spinning subsequently is a necessary step and should never be skipped or you will lose your culture.

Freezing medium:

Growth medium with 10%DMSO or 10% glycerol.