HiB5 Care

Method:

1. To split plates remove media, add 3 ml of trypsin-EDTA.

2. Observe under the microscope for a few minutes until they begin to retract and come off the plate. Remove the trypsin-EDTA and replace with growth media (4 ml/ 60 mm plate).

3. Agitate flasks until cells are in solution, split volume between desired number of flasks/dishes. Supplement with media to desired final volume.

Recipes:

Coat cells with PL/laminin in usual fashion.

Media: DMEM supplemented with 0.11 g/L NaPyruvate; 3.7 g/L $NaHCO_3$; 0.29 g/L glutamine; 3.9 g/L HEPES; Pen/Strep and 10%v/v FCS.

Notes:

The cells have been transformed with the temperature-sensitive allele tsA58 of the SV40 large T antigen; thus the cells remain undifferentiated at 33C and differentiate at 37C.