

Mouse NPC Protocol

Billiar Lab SOPs

Mouse NPC Protocol

Yield: approximately 60-100 million cells/mouse

All cells are NPC cells: Kuppger (largest), endothelial cells, and stellate cells (smallest)

DO NOT gelatin coat wells/plates for NPCs if using without HPCs as they are adherent

Harvest Procedure

Start by following same perfusion sequence as for Mouse Hepatocytes, but after the first 2 minute spin at 400 rpm save the supernatant. This is considered Spin 1.

Spin 2: Using supernatant from Spin 1, spin at 400 rpm for 5 minutes. (Spin 1 pellet contains hepatocytes)

Spin 3: Using supernatant from Spin 2, spin at 1000 rpm for 5 minutes (Discard Spin 2 pellet)

Spin 4: Discard supernatant from Spin 3, save pellet. Re-suspend pellet in serum free media and spin at 1000 rpm for 5 minutes

Count cells after re-suspending pellet from Spin 4 (discard supernatant) in 5-10% Calf serum media.

Plate using a 5:1 concentration NPCs to HPC

Ex: HPC for 6 well is 2.4×10^6 so NPC would be 1.2×10^7 for the total plate or 4×10^5 HPC/well and 2×10^6 NPC/well