**Macrophage collection for Hybridoma thawing**

🡪 Prepare at least two mice per hybridoma thawing: you will prepare 1x 24-well plate per mouse. If you can have more mice (and thus more frozen hybridoma), it’s worth trying because contaminations happen often.

**! Never mix the macrophages from different mice**

**to avoid cross contamination !**

Material for **one** mouse:

* One 24-well plate (prepare 10 wells)
* 5 mL of 1X sterile PBS pre-warmed @ 37°C
* 10 mL of complete Hybridoma medium (such as IMDM) pre-warmed @ 37°C
* 10mL syringe and 21G needle
* one sterile 15 mL tube

Procedure :

* Pre-warm 1X PBS and medium for hybridoma @ 37°C
* Sacrifice a mouse following the ethical rules
* Under a microbiological hood, wash the mouse with 70% Ethanol
* Cut and open the skin to have access to the peritoneum
* Inject 5 mL of prewarmed PBS into the peritoneum. Don’t remove the needle
* Massage the peritoneum
* Recover the PBS/macrophages with the syringe and load it into the 15 mL tube
* Centrifuge 10 minutes at 1000 g at room temp
* Resuspend the macrophages pellet into 10 mL of pre-warmed hybridoma medium
* Plate 1mL of macrophage per well into the 24-wells plate.
* Incubate @ 37°C 5% CO2 overnight and check for contamination
* If not contamination, add directly onto the macrophages 50, 100, 150, 200, 250ul, rest of the thawed hybridoma.
* When the hybridoma is growing, you can transfer onto 6-well plate also with macrophages (prepare fresh macrophages 4ml per well, check first for contamination).

**IMDM HT medium :**

ISCOVE : Iscove’s modified dulbecco’s medium (Sigma #I7633) 100 ml

Resuspend 17,7 g into 950 ml H2O.

Store at 4°C.

Kanamycine (25 mg/ml in H2O) 250 ul

Sodium bicarbonate (NaHCO3, Sigma #S-6014) (3,24 g / 50 ml H2O) 5 ml

**HT (50x) 2 ml**

L-Glutamine (Sigma #G-7029) (2% in H2O) 1 ml

Sodium Pyruvate (Sigma #P-3662) (1M in H2O) 200 ul

2--Mercaptoéthanol (Sigma #M-6250) (0,1 M, dilute 143 times into H2O) 50 ul

FCS (heated 30 min at 56°C) 10 ml

**HT : Sigma #H0137**: Hypoxanthine—Thymidine (HT) media supplement **50X** (stored at –20°C). Reconstitute in 10 ml Iscove (50x).