1. Sample collection (healthy donors).
2. Isolation of PBMCs (phicoll hypaque).
3. Separation of monocytes → plastic adherence (incubation 2 hours 37°C).
4. Wash the non-adherent cells away.

Test different culturing medias

Control Group 1

- Adherent cells
- Supernatant

Harvest

CD markers
- CD14
- CD1a
- CD83
- CD86
- CCR7

(Baseline readings)

Blast cell collection

Freez and Thaw / UV

Tumor lysate preparation

5 days culture (GM-CSF, IL-4)

iDC

+ lysate loading 3 hours/37°C

Group 2

- CD markers
  - CD14
  - CD1a
  - CD83
  - CD86
  - CCR7

Group 3

- + TNFα
- IL-1β
  [CC]

(-) MPLA

48 hours incubation

Cells

Supernatant

Stored

For IL-10
IL-12
ELISA

Test different culturing medias