

## MICROGLIAL CULTURES

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Saura J, Tusell JM, Serratosa J. High-yield isolation of murine microglia by mild trypsinization. *Glia*. 2003 Dec;44(3):183-9.

Once the mixed cultures are confluent, wait at least 3-4 more days (5-10 days even better) then do the mild trypsinization step to isolate microglia:

- Remove medium from cells (do not discard it! Keep it warm).
- Incubate cells at 37°C with “mild trypsin” (Trypsin-EDTA (GIBCO, #25200-072)+DMEM-F12 (GIBCO, #31330-038), 1:3. After 20-30min the astroglial layer will detach.
- When astroglial layer is fully detached, add DMEM-F12+10% FBS to stop trypsin activity.
- Aspirate medium and discard astroglial layer.
- Add conditioned medium from step 1.

Use microglial cells preferably 24 hrs after mild trypsinization.