BRL-CONDITIONED MEDIUM

1. T175 flasks

- Seed one vial of Buffalo Rat Liver (BRL) cells onto one T175 in 40 ml of complete medium.
- When confluent (after 3 days), passage cells to 10 T175 flasks:
  - wash cells once with 5 ml PBS.
  - add 5 ml of Trypsin/EDTA, incubate for 5 min at 37 °C.
  - add 5 ml of complete medium, resuspend cells and transfer 10x 1ml to 10xT175 with 40 ml of complete medium.
- When confluent refeed cells with 40 ml of complete medium and incubate for 3 days.
- Start conditioning:
  - refeed cells with 60 ml of complete medium.
  - incubate cells for 7 days.
  - harvest medium, filtrate through 0.8 μm Millex-AA and store 150 ml portions at -20 °C.
  - repeat 4 times with fresh complete medium.

  NOTE : check cells each time under the microscope for infection and confluency.

2. Roller bottles

- Grow BRL cells to confluency on 8-10 T175 flasks as described above.
- Trypsinize cells with 5 ml Trypsin/EDTA per flask.
- Neutralize with 5 ml of complete medium.
- Resuspend cells from one T175 and transfer to one roller bottlle (surface 850 cm^2) containing 150 ml of complete medium.
- Gaz bottles with 5% CO_2 for 2 min and close.
- Roll at 37 °C till confluency (7 days).
- Start conditioning:
  - refeed cells with 300 ml of complete medium, gaz for 2 min and close.
  - roll bottles for 7 days at 37 °C.
  - harvest medium, filtrate through 0.22 μm and store 150 ml portions at
    -20°C.
  - repeat 4 times with fresh complete medium.

  NOTE : check cells each time under the microscope for infection and confluency.