

B cell infection protocol (Tao Gan)

Cells:

- B220+ enriched BM cells (using B220 microbeads from Miltenyi)
- Or better, sorted pro-B cells from BM (CD43+/B220+/lineage-negative, PI-negative)

Infection medium:

2X Opti-MEM (GIBCO REF 31985-070) based medium:

recipe for 50 mL:

- 20% FBS (Gibco/Life Technologies) =10ml
(REF 1047-028 LOT 822043)
- 0.36 μ L 2-ME (stock=14.3M)
- 1 mL 2% L-Glutamine (stock = 200mM)
- Antibiotics optional
- Opti-MEM Up to 50 mL
- Filter 0.2 micron is also optional

Infection set-up:

1. Resuspend B cells in 2x infection medium and add equal volume of virus supernatant, mix well.
2. Add recombinant murine IL-7 (PeproTech 217-17) to 5 ng/mL and polybrene (Sigma) to 4 μ g/mL
3. Spin-infect for 90 minutes at 2500 rpm (1398 x g), 32°C
4. After spin infection, incubate with virus for another 2-3 hours at 37°C (this step is optional) before spinning down and switching to fresh medium (1x opti-MEM medium containing all of the components above in the recipe including 5 ng/mL IL-7, but diluted to 1x. Do not add polybrene).
5. Incubate for another 2 days and check infection efficiency. We make our viral supernatants by cotransfection; decent supernatants should give 70-100% infection efficiency with this protocol.