# Naïve T Lymphocyte Infection by Murine Stem Cell Virus (MSCV) Protocol

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**[Abstract]** This assay can be used for studying genes related to lymphocyte proliferation and differentiation *in vitro*. Stimulating lymphocytes to proliferate is important for the infection efficiency of MSCV. After overnight culture, if naïve cells proliferate extensively, the following spin infection will have high efficiency. After infection, cells are ready for the following assay as they will be undergoing proliferation or differentiation.

## Materials and Reagents

1. Mouse
2. Fetal bovine serum (FBS)
3. Anti-CD3 and Anti-CD28 antibody (Ab) (Biolegend, catalog number: 100302, 102102）
4. IL-2 (R&D systems, catalog number: 402-ML-020)
5. Polybrene (Hexadimethrine bromide) (Sigma-Aldrich, catalog number: H9268)
6. Fetal bovine serum (FBS) (Gemini Bio-Products, catalog number: 900-108)
7. Penicillin/streptomycin solution (Life Technologies, Gibco®, catalog number:15140-122)
8. 2-mercaptoethanol (Life Technologies, Invitrogen™, catalog number: 21985-023)
9. PBS solution (1x phosphate buffered saline) (Life Technologies, Gibco®, catalog number: 10010-023)
10. MSCV (Clontech, catalog number: 634401)
11. RPMI-1640 medium (Life Technologies, Gibco®, catalog number: 11875-093) (see Recipes)

## Equipment

1. Centrifuges
2. 24-well plate
3. Water bath
4. Parafilm

## Procedure

1. Coat plate with anti-CD3 and anti-CD28 Ab overnight.
   Add 400 µl/well PBS containing 1 µg $ml^{-1}$ of anti-CD3 and 0.5 µg $ml^{-1}$ of anti-CD28 to 24-well plate. Place lid or seal with parafilm, and keep in dark 4 °C overnight. Next day, discard the antibodies by aspiration and add 400 µl/well of RPMI-1640 culture medium; do not let the plate dry.
2. Sacrifice mouse and sort CD4+CD44 loCD62Lhi naïve T cells.
3. Seed 5 x $10^6$ cells/well for 24-well plate in PRMI-1640 culture medium and culture cells overnight.
4. Spliting cells in the ratio 1: 2 (separate one well cells into two wells), adding IL-2 at final concentration of 50 U $ml^{-1}$, culture for 6 h (37 °C, 5% $CO_2$).
5. Spin the plate at 1,200 RPM for 5 min (room temperature), remove culture medium by aspiration, and add 1 ml RPMI-1640 with proper amount of MSCV particles (virus concentration depends on the titter which is more than $10^6$ cfu $ml^{-1}$) and 5-10 µg $ml^{-1}$ of polybrene. Spin the plate at 2,500 RPM (37 °C) for 90 min.
6. Remove supernatant and add RPMI-1640 culture medium for differentiation or proliferation.

## Recipes

1. RPMI-1640 culture medium
   Supplemented with
   5% FBS
   1% Penicillin/Streptomycin solution
   50 µM 2-mercaptoethanol