# EGFP Retrovirus

**Store at -80°C**

| Cat. No. | Description | Quantity |
|---|---|---|
| C014 | EGFP Retrovirus ($1X10^6$ ipu/ml) | 20.0ml |

## Description

The recombinant retroviral vectors have been used to transduce many mammalian cell types (dividing cells) for efficient gene expression of the recombinant proteins. Recombinant retroviral vector are especially useful for gene transfer and protein expression in cells that have low transfection efficiency with other transfection reagents. After entering cells, the virus will integrated into host cell genome and produce a stable expressing cell line. Retrovirus containing EGFP gene can be used as positive control for evaluating infection efficiency in target cells.

## Positive Target Cells

The quality of our EGFP positive control retroviruses have been tested on control target MDA-MB-468 cells which will give upto 80-90% infection efficiency 2-3 days post viral infection.

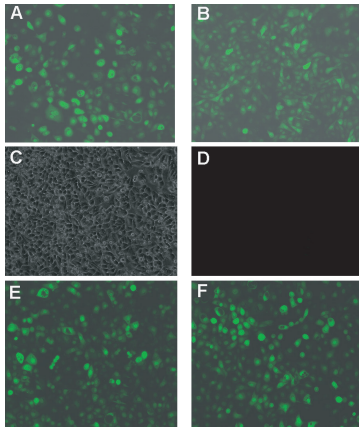


Figure. MDA-MB-468 cells infected with EGFP retrovirus. Cells were infected with freshly prepared viral supernantant (E, F), or thawed frozen viral supernantant (A, B). No signals were visible in uninfected MDA-MB-468 cells (C, D).

## Retroviral and Lentiviral Infection of Target Cells (Protocol)

1. Thaw the recombinant retrovirus supernatant in a 37°C waterbath and remove it from the bath immediately when thawed.

2. Prepare polybrene stock to a concentration of 0.8mg/ml.

3. In the early morning, infect the target cells in a 6-well plate with 2ml/well supernatant in the presence of 8µg/ml polybrene (add 20µl of the stock polybrene to 2.0ml of the viral supernatant, 1:100 dilution). Place the remainder of the viral supernatant in the fridge for the second infection in the afternoon.

4. 6-8 hours later, remove the viral supernatant (from the first infection) from the wells and re-infect the cells with 2.0ml of fresh supernatant (with polybrene).

5. For Lentiviral vector, one infection (incubate over night) works well for most target cells. Dilute Lentiviral vector with fresh complete medium (1:1) if cytotoxicity is a problem.

6. The next day, remove viral supernatant and add the appropriate complete growth medium to the cells and incubate at 37°C.

7. 48-72 hours after incubation, sub-culture the cells into 2 x 100mm dishes and add the appropriate selection drug for stable cell-line generation.

8. For the EGFP retrovirus, the selection marker is Puromycin. For most cell lines the selection concentration is between 0.2 - 1.0µg/ml (often around 0.3µg/ml).

9. 10-15 days after selection, pick clones for expansion and screen for postive ones.

NOTES:

- After thawing, we recomnend that the supernatant not be frozen again for future use since the virus-titer will decrease significantly.

- Infection of MDA-MB-468 cells would be a good control for the EGFP-virus.



CERTIFICATE OF ANAYLSIS