Virongy Biosciences
Accelerate Discovery

Virongy HIV Rev-dependent Reporter Cells

A new generation of HIV indictor cells

The HIV Reve dependent reporter cells represent a major advancement in the development of HIV indicator cells (Wu et al., 2007). This new reporter system differs dramatically from the common LTRe based reporter cells, which rely solely on the HIV promoter, the long terminal repeat (LTR), to drive reporter expression. While responsive to an early HIV protein, Tat, the LTR is also responsive to cell culture conditions and stimulation by a variety of known and unknown factors, including cytokines, mitogens, HDAC inhibitors, lipopolysaccharide, certain antie tumor drugs, and free viral proteins (Siekevitz et al., 1987; Sweet et al., 1995). Such none HIVe dependent reporter expression frequently diminishes reporter specificity and sensitivity. In contrast to the LTRe based reporter cells, our Reve dependent reporter cells use both LTR and the Rev/RRE interaction to regulate reporter gene expression. This strict requirement for Rev, a viral protein present only in infected cells, drastically improves the reporter specificity and sensitivity. As a result, our Reve dependent reporter cells are suitable for a broad range of applications, including screening broadly neutralizing antibodies and antie HIV drugs, and studying HIV celle cell transmission and host restriction and dependency factors. Derived from CD4 T cells, our reporter cells express native levels of HIV receptors and are natural HIV targets with broad susceptibility to X4, R5, primary HIV isolates, and certain SIV strains. With GFP, Luc, or GFP/Luc detection options, our Reve dependent cells provide a versatile and flexible platform for HIV research.

Highlights

- Unparalleled sensitivity & specificity: Rev-regulated reporter expression
- Versatile: GFP & Luciferase dual reporter system
- Natural HIV target: Derived from human CD4 T cells
- Physiologically relevant: Natural levels of HIV receptors/co-receptors
- Broad susceptibility: Susceptible to X4, R5, primary HIV isolates, some SIV

Applications

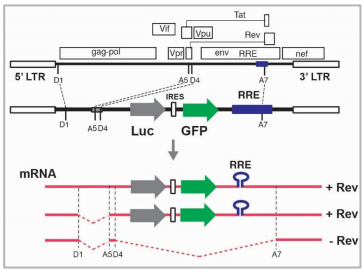
Our Rev-dependent reporter cells allow you to easily perform:

- TCID50 assays: Routine HIV infectivity & quantification
- Anti-HIV drug screenings via one-step infection
- Routine EC50/LD50 quantifications of anti-HIV compounds
- Screenings for broadly neutralizing antibodies (bnAB) (from laboratory and clinical research samples)
- Neutralizing antibody quantifications
- HIV cell-cell transmission and HIV drug-resistance studies
- HIV host restriction factor (HRF) studies
- HIV host dependency factor (HDF) studies
- Low-level HIV gene expression assessments
- HIV pre-integration transcription studies
- HIV latency and reactivation studies
- HIV outgrowth detection following reactivation
- HIV tropism determinations

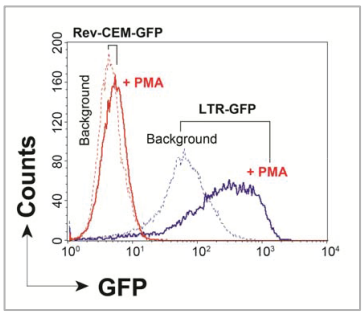
Virongy LLC, 9385 Discovery Blvd. Lab 119, Manassas, VA 20109, U.S.A.
Email: Info@virongy.com Tel: (703) 257 5500

Greater HIV Sensitivity and Specificity – Minimal Background Signal

Nearly all HIV indicator cells use the LTR promoter to drive reporter expression. While the LTR promoter is responsive to HIV Tat, it generates false positive reporter expression due to HIV-independent factors, resulting in lower HIV specificity and a lower HIV detection range. Changes in cell culture conditions and the presence of mitogens, cytokines, cellular activators, and chromatin modulators can all produce background signal in LTR reporters. Our Rev-dependent reporter cells overcome the drawbacks of LTR reporter lines by being engineered to use the interaction between HIV Rev and RRE (Rev-Response Element) to regulate reporter expression. Rev is present only in HIV+ cells. The high stringency that Rev imposes on the reporter dramatically decreases background signal and significantly increases sensitivity. Thus, our Rev-dependent reporter cells specifically detect low levels of HIV replication even in the presence of environmental factors that generate false signal with the LTR promoter.



Our Rev-dependent reporter cells carry stably integrated reporter constructs that are derived from the HIV genome. The incorporation of RRE and multiple, authentic HIV splicing sites permits reporter expression only from the non-spliced and singly-spliced transcripts in the presence of Rev.

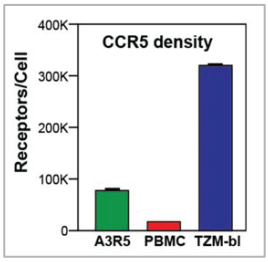


Our Rev-dependent reporter cells are highly HIV-specific when compared with the LTR-GFP indicator cells. Without HIV, our reporter cells have undetectable GFP, whereas the LTR-GFP cells have high background GFP. Our Rev-dependent reporter cells do not respond to PMA stimulation (100 ng ml^{-1}), while the LTR-GFP cells respond to PMA stimulation by producing high levels of GFP signal in the absence of HIV.

Thoroughly Characterized

Each of our cell lines has been thoroughly characterized and validated with regards to HIV responsiveness and sensitivity to anti-HIV inhibitors, HIV activators, and HIV neutralizing antibodies. Our reporter cells are derived from human T cells and carry physiological or near-physiological levels of HIV receptors and relevant T-cell receptors. As such, our reporter cells are especially suited for quantifying HIV isolates and bnABs using laboratory and clinical research samples.

Greater Physiological Relevance



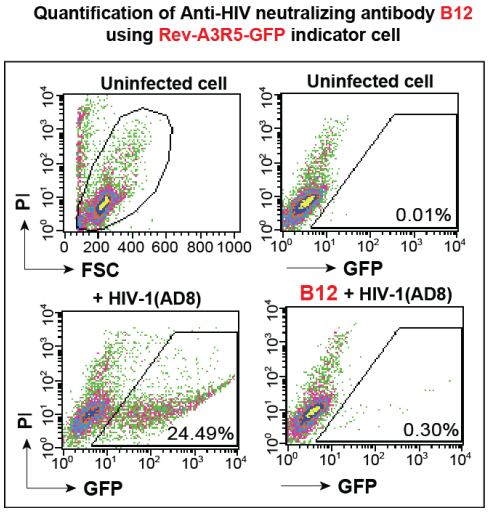
Our Rev-dependent reporter cells are derived from CD4 T cells such as CEM-SS (Wu et al., 2007) and A3R5 (McLinden et al., 2013), and are further engineered with a dual LTR and Rev-dependent reporter system. Because they are derived from CD4 T cells and do not contain a superabundance of HIV receptors, our reporter cells provide a physiologically relevant and nAB-sensitive reporter system.

Receptor cell density of A3R5 cells. Source: PLOS ONE, McLinden et al. 2013, 8: 11, e7756. Our Rev-dependent Cell Lines (derived from CEM-SS & A3.01 cells) do not have a superabundance of receptors and more closely mimic natural T-cell HIV receptor densities

Virongy LLC, 9385 Discovery Blvd. Lab 119, Manassas, VA 20109, U.S.A.
Email: Info@virongy.com Tel: (703) 257 5500

Examples of Application

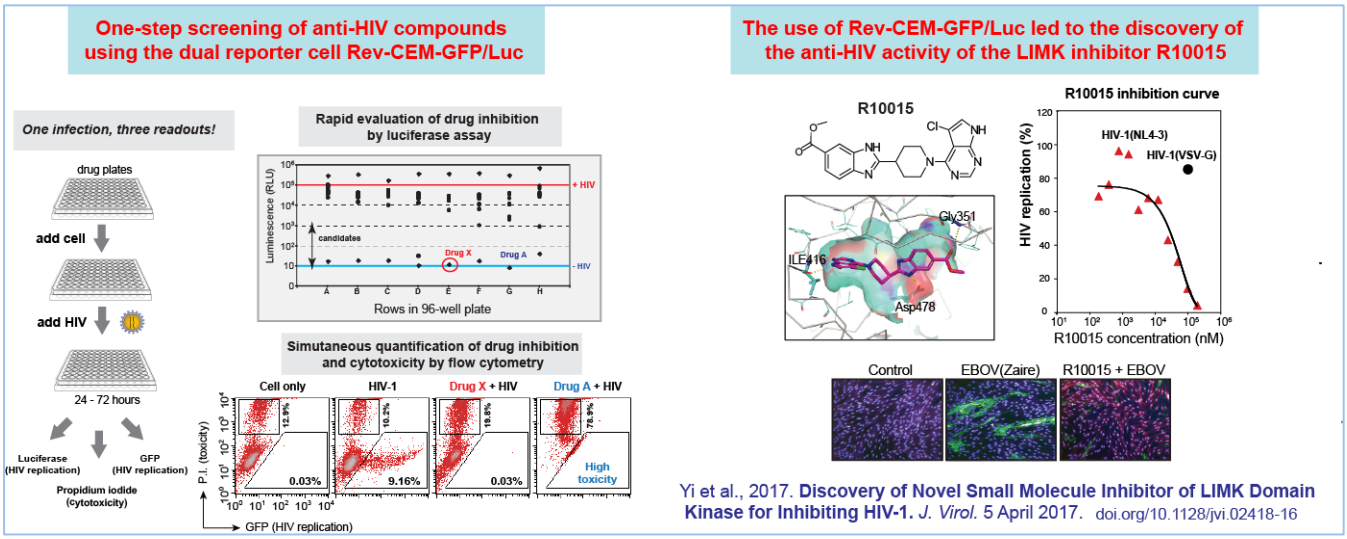
Quantification of HIV-neutralizing antibodies



Prior to infection, HIV(AD8), an R5 virus, was incubated with or without the HIV neutralizing antibody B12 (10 µg ml^{-1} final). After 1 h, Rev-A3R5-GFP cells were infected with Ab-neutralized and non-neutralized virus. Cells were washed and cultured for 48 hours. GFP expression was quantified by flow cytometry. PI = propidium iodide.

One Step Anti-HIV Drug Screening with the Rev-dependent GFP/Luc Reporter Cell

Easy to Use: One Infection, Three Readouts. Increase the efficiency of your screening process by simplifying your protocol. With our Rev-dependent reporter cell system, three readouts may be generated with one infection. Easily screen for positive candidates based on the luciferase signal and then obtain population dynamics through flow cytometry based on the fluorescent signal of GFP and/or a vital dye.



Yi et al., 2017. **Discovery of Novel Small Molecule Inhibitor of LIMK Domain Kinase for Inhibiting HIV-1.** *J. Virol.* 5 April 2017. doi.org/10.1128/jvi.02418-16

The Making of the Rev-dependent Reporter Cells

The HIV Rev-dependent Reporter cell lines were originally developed by Wu & Marsh at the National Institutes of Health. The first generation of the Rev-dependent cell, Rev-CEM, has been used extensively in multiple laboratories for studying HIV infection, anti-HIV drugs, and HIV cell-cell transmission. Multiple Rev-dependent Reporter cells have been developed recently to meet the needs of the HIV/AIDS research community.

Virongy LLC, 9385 Discovery Blvd. Lab 119, Manassas, VA 20109, U.S.A.
Email: Info@virongy.com Tel: (703) 257 5500

Protocol

HIV Infection of Rev-dependent reporter cells

(When using HIV Rev-dependent Reporter cells, use of HIV Infectin™ is required for optimal results)

1) Count cells to be infected, and pellet cells by centrifugation at 300 x g for 5 minutes.
Note: Cell viability should be ≥ 80%.
2) Resuspend cells in complete media (RPMI 1640) at a concentration of ~2 x 10^6 cells ml^{-1}.
3) Use 100 µl of cells (~2 x 10^5 cells) per infection.
4) Pre-treat cells by adding 10 µl of HIV Infectin™ (10X) so that the HIV Infectin™ concentration is 1X. Mix and incubate for 2 hours. (When using HIV Rev-dependent Reporter cells, use of HIV Infectin™ is required for optimal results)
5) Add virus to the cells and mix. Note volume of virus used.
6) Add HIV Infectin™ (10X) in an amount equal to 1/10 of the virus volume used. For example, if 100 µl of virus is used, add 10 µl of HIV Infectin™. Incubate at 37°C for 2–4 hours.
7) Wash cells by adding an additional 1 ml fresh complete media, pelleting cells as in 1) above, and removing supernatant. (Optional) Repeat once for a total of two washes.
8) After washing, resuspend cells in 1 ml complete medium.
9) Culture and utilize cells as usual.

References

Wu Y, Beddall MH, Marsh JW. Rev-dependent indicator T cell line. Current HIV Research. 2007; 5:395-403.

Siekevitz M, Josephs SF, Dukovich M, Peffer N, Wong-Staal F, Greene WC. Activation of the HIV-1 LTR by T cell mitogens and the transactivator protein of HTLV-I. Science. 1987; 238:1575–1578.

Sweet MJ, Hume DA. RAW264 macrophages stably transfected with an HIV-1 LTR reporter gene provide a sensitive bioassay for analysis of signalling pathways in macrophages stimulated with lipopolysaccharide, TNF-alpha or taxol. J Inflamm. 1995; 45:126 –135.

Yu D, Wang W, Yoder A, Spear M, Wu Y. The HIV envelope but not VSV glycoprotein is capable of mediating HIV latent infection of resting CD4 T cells. PLoS Pathog. 2009; 5(10):e1000633. PubMed PMID: 19851458.

Sigal A, Kim JT, Balazs AB, Dekel E, Mayo A, Milo R, et al. Cell-to-cell spread of HIV permits ongoing replication despite antiretroviral therapy. Nature. 2011; 477(7362):95-8. PubMed PMID: 21849975.

Yoder A, Yu D, Dong L, Iyer SR, Xu X, Kelly J, et al. HIV envelope-CXCR4 signaling activates cofilin to overcome cortical actin restriction in resting CD4 T cells. Cell. 2008; 134(5):782-92. PubMed PMID: 18775311.

Spear M, Guo J, Turner A, Yu D, Wang W, Meltzer B, et al. HIV-1 triggers WAVE2 phosphorylation in primary CD4 T cells and macrophages, mediating Arp2/3-dependent nuclear migration. J Biol Chem. 2014; 289(10):6949-59. PubMed PMID: 24415754; PubMed Central PMCID: PMC3945356.

Sloan RD, Kuhl BD, Donahue DA, Roland A, Bar-Magen T, Wainberg MA. Transcription of preintegrated HIV-1 cDNA modulates cell surface expression of major histocompatibility complex class I via Nef. J Virol. 2011; 85(6):2828-36. PubMed PMID: 21209113.

Shuck-Lee D, Chang H, Sloan EA, Hammarskjold ML, Rekosh D. Single-nucleotide changes in the HIV Rev-response element mediate resistance to com-pounds that inhibit Rev function. J Virol. 2011; 85(8):3940-9. PubMed PMID: 21289114.

Guo J, Wang W, Yu D, Wu Y. Spinoculation triggers dynamic actin and cofilin activity facilitating HIV-1 infection of transformed and resting CD4 T cells. J Virol. 2011; 85(19):9824-33. PubMed PMID: 21795326.

VIRONGY

Virongy.com

Rev-dependent Reporter Cells and Infection Medium

Catalog No.	Product	Description	Size	Price[1]
RevCell-01	Rev-A3R5-GFP	Derived from A3.01 cells. Natural CD4, CXCR4, and α4β7 expression. Constitutive CCR5 expression. Rev-dependent GFP expression.	5 X 10^6 cells/vial	$1,399
RevCell-02	Rev-A3R5-GFP/Luc	Derived from A3.01 cells. Natural CD4, CXCR4, and α4β7 expression. Constitutive CCR5 expression. Rev-dependent GFP and Luc expression.	5 X 10^6 cells/vial	$1,899
RevCell-03	Rev-A3-GFP/Luc	Derived from A3.01 cells. Natural CD4, CXCR4, and α4β7 expression. Rev-dependent GFP and Luc expression.	5 X 10^6 cells/vial	$1399
RevCell-04	Rev-CEM-GFP	Derived from CEM-SS cells. Natural CD4 and CXCR4 expression. Rev-dependent GFP and Luc expression.	5 X 10^6 cells/vial	$899
RevCell-05	Rev-CEM-GFP/Luc	Derived from CEM-SS cells. Natural CD4 and CXCR4 expression. Rev-dependent GFP and Luc expression.	5 X 10^6 cells/vial	$999
RevCell-06	Rev-CEM-Luc	Derived from CEM-SS cells. Natural CD4 and CXCR4 expression. Rev-dependent Luc expression.	5 X 10^6 cells/vial	$899
HI-01	HIV Infectin™	HIV infection medium for Rev-dependent cells	200 µl	$120
HI-02	HIV Infectin™	HIV Infection medium for Rev-dependent cells	500 µl	$250
HI-02	HIV Infectin™	HIV Infection medium for Rev-dependent cells	1 ml	$480
HI-03	HIV Infectin™	HIV Infection medium for Rev-dependent cells	5 ml	$2180
[1]Academic and government price. Others please inquire.				
Technical Support Email: info@virongy.com				

* HIV Rev-dependent Reporter Cells may

For order:

Virongy.com
Email: info@virongy.com
Tel: (703) 257-5500