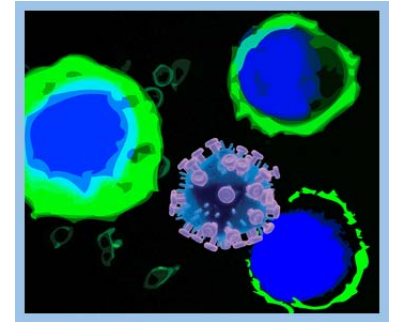


# HIV Reporter Cell System

Are your current cells leaving you *blue*?

## A new generation of HIV Reporter Cells

- Unparalleled **sensitivity & specificity**: Rev-regulated reporter
- **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



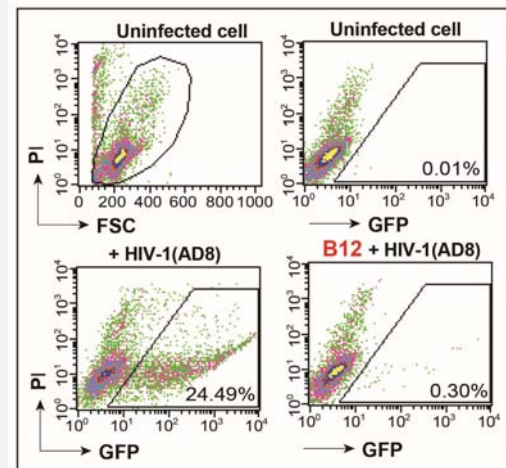
## ViroVision™ Applications

ViroVision™ cells allow you to more easily perform:

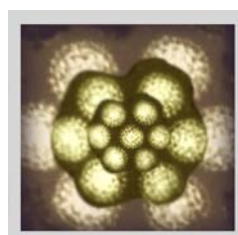
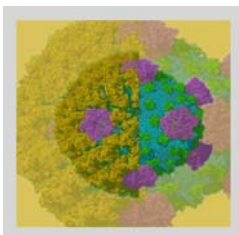
- ◆ TCID50 assays: Routine HIV infectivity & quantification
- ◆ Anti-HIV drug screenings via one-step infection
- ◆ Routine EC50/LD50 quantifications of anti-HIV compounds
- ◆ Screenings for neutralizing antibodies (bnAB) (from laboratory and clinical research samples)
- ◆ Neutralizing antibody quantifications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

### Application:

#### Quantification of HIV neutralizing antibodies



Prior to infection, HIV(AD8), an R5 virus, was incubated with or w/o the HIV neutralizing antibody B12 ( $10 \mu\text{g ml}^{-1}$  final). After 1 h, ViroVision™ 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.

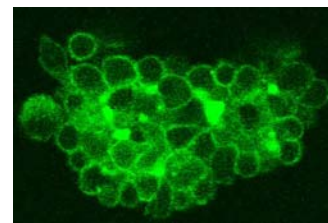
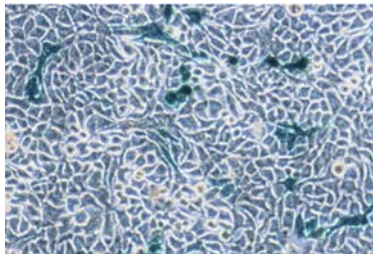


## Tired of Being Blue?

Go **Green** or **Firefly** Instead...

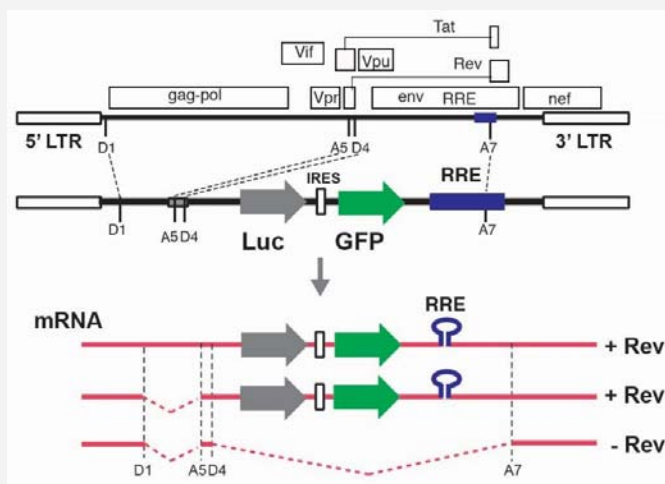
Have you ever mis-timed your assays only to have all your cells turn blue? Does the picture look all too familiar? ViroVision™ Cells eliminate the potential to waste time and money because your current indicator cells turned blue without HIV infection or because you

did not perfectly time your X-Gal staining. With unparalleled **specificity & sensitivity**, ViroVision™ Cells come with a GFP, Luciferase or a combination GFP/Luciferase reporter that provides you with ease of use and flexibility.

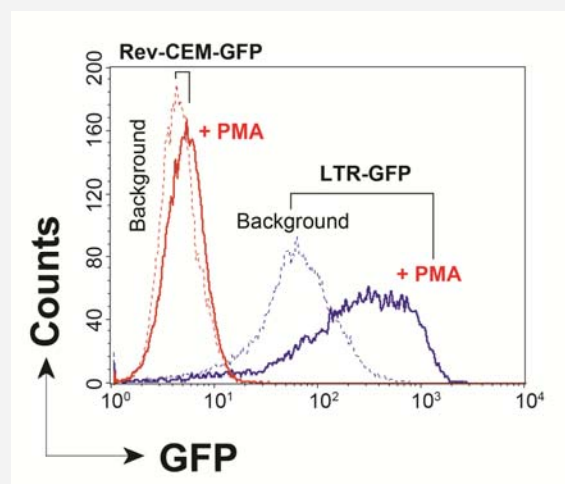


## Greater HIV **Sensitivity and Specificity**— Minimal background signal

Nearly all HIV Indicator Cells use the LTR promoter to drive reporter expression. While responsive to HIV Tat, the LTR promoter 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, the presence of mitogens, cytokines, cellular activators, and chromatin modulators can all produce high background signal in LTR reporters. ViroVision™ 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, ViroVision™ Reporter Cells accurately detect low-levels of HIV activity even in the presence of environmental factors that generate signal with the LTR promoter.



ViroVision™ 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.

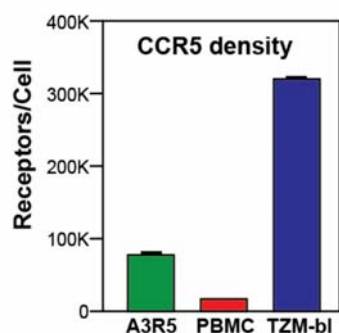


ViroVision™ Cells are more HIV-specific when compared with LTR-GFP indicator cells. Without HIV, ViroVision™ Cells have undetectable GFP, whereas LTR-GFP cells have high background GFP. ViroVision™ Cells do not respond to PMA stimulation ( $100 \text{ ng ml}^{-1}$ ). For LTR-GFP cells, PMA induces high levels of GFP signal.

## Thoroughly Characterized

Each ViroVision™ cell line has been thoroughly characterized and validated with regards to HIV responsiveness and sensitivity to anti-HIV inhibitors, HIV activators, and HIV neutralizing antibodies. ViroVision™ 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, ViroVision™ cells are especially suited for quantifying HIV isolates and bnABs using laboratory and clinical research samples.

## Greater Physiological Relevance



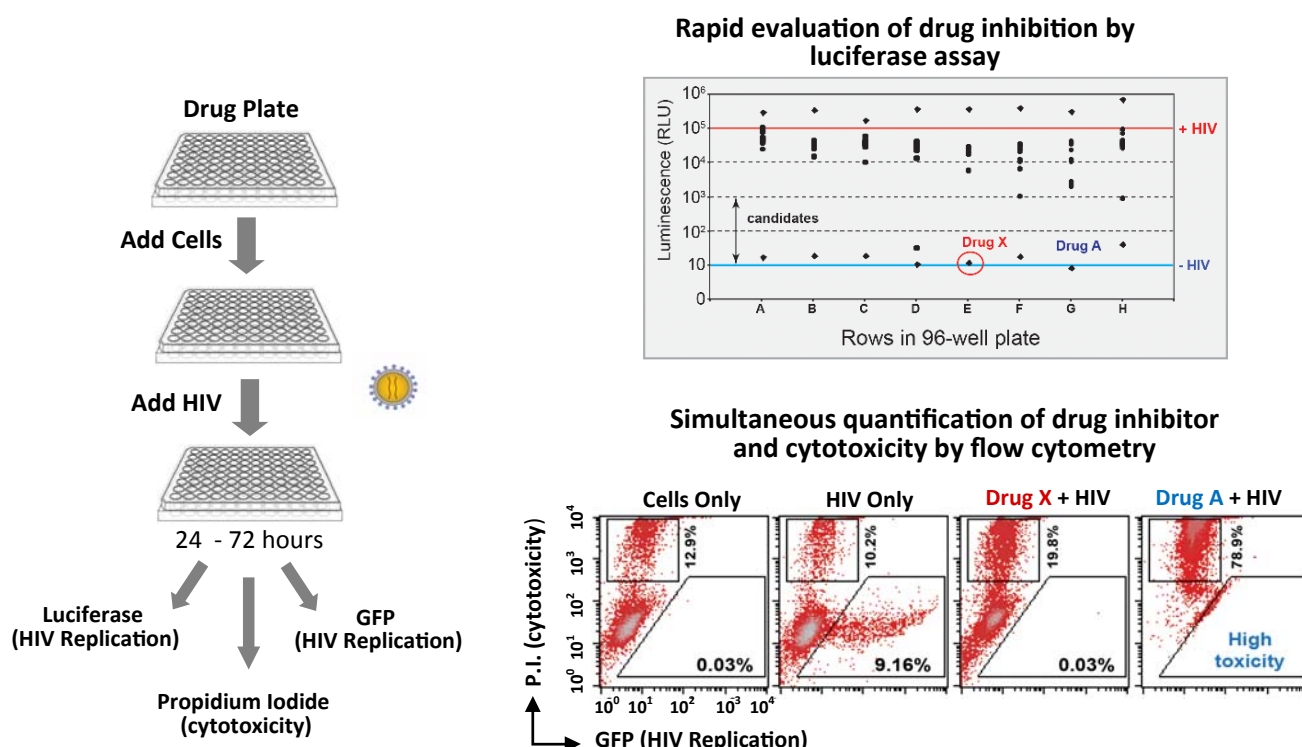
ViroVision™ Cells are derived from CD4 T-cells [CEM-SS (Wu et al, 2007) & A3R5, (McLinden *et al.* 2013)] and further engineered with a dual LTR and Rev-dependent reporter system. Because they are derived from CD4 T-cells and do not contain a super-abundance of HIV receptors, the ViroVision™ cells provide a more **physiologically relevant** and nAB sensitive reporter system.

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

## Easy-to-Use: One Infection, 3 Readouts

Increase the efficiency of your screening process by simplifying your protocol. With the ViroVision™ System, 3 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.

### One Step Anti-HIV Drug Screening with the ViroVision™ Rev-GFP/Luc Cell System





# ViroVision™ HIV Reporter Cells

## Ordering ViroVision™ Cells

Product	Catalog	Description	Size
Rev-A3R5-GFP Cells	CUBRC0011	Derived from A3.01 cells. Natural CD4, CXCR4 and $\alpha 4\beta 7$ expression. Constitutive CCR5 expression. Rev-dependent GFP expression.	5 X 10 <sup>6</sup> cells/vial
Rev-A3R5-GFP/Luc Cells	CUBRC0012	Derived from A3.01 cells. Natural CD4, CXCR4 and $\alpha 4\beta 7$ expression. Constitutive CCR5 expression. Rev-dependent GFP and Luc expression.	5 X 10 <sup>6</sup> cells/vial
Rev-A3-GFP/Luc	CUBRC0022	Derived from A3.01 cells. Natural CD4 and CXCR4 expression. Rev-dependent GFP and Luc expression.	5 X 10 <sup>6</sup> cells/vial
Rev-CEM-GFP	CUBRC0031	Derived from CEM-SS cells. Natural CD4 and CXCR4 expression. Rev-dependent GFP and Luc expression.	5 X 10 <sup>6</sup> cells/vial
Rev-CEM-GFP/Luc Cells	CUBRC0032	Derived from CEM-SS cells. Natural CD4 and CXCR4 expression. Rev-dependent GFP and Luc expression.	5 X 10 <sup>6</sup> cells/vial
Rev-CEM-Luc Cells	CUBRC0033	Derived from CEM-SS cells. Natural CD4 and CXCR4 expression. Rev-dependent Luc expression.	5 X 10 <sup>6</sup> cells/vial

## The Making of ViroVison™ 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 in Wu's lab at George Mason University to meet the needs of the HIV/AIDS research community.

### References:

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

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 compounds 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.

**ViroVison™ HIV Reporter Cells only may be obtained with a Limited Use License. ViroVison™ HIV Reporter Cells are intended for Research Use Only and are not for diagnostic or therapeutic purposes or uses in humans or animals.**