CELLECTA

USER MANUAL

PromoterTest™ Custom Assay Kit

v1 — Last update: 19 January 2024

Cellecta, Inc.

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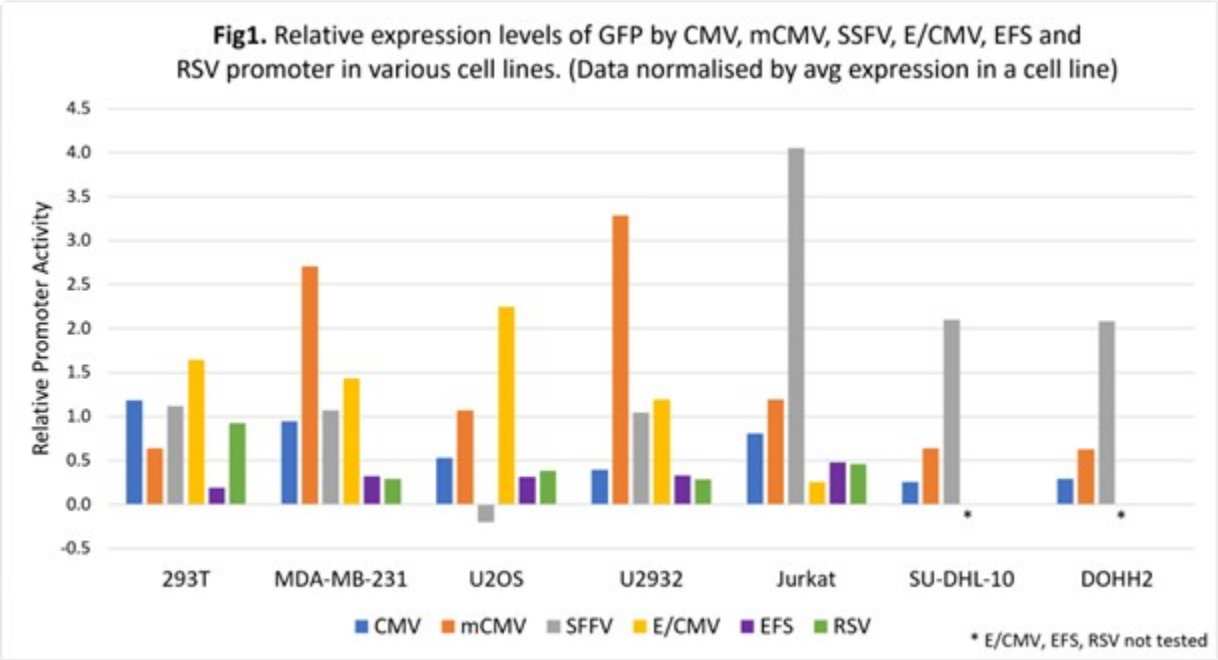
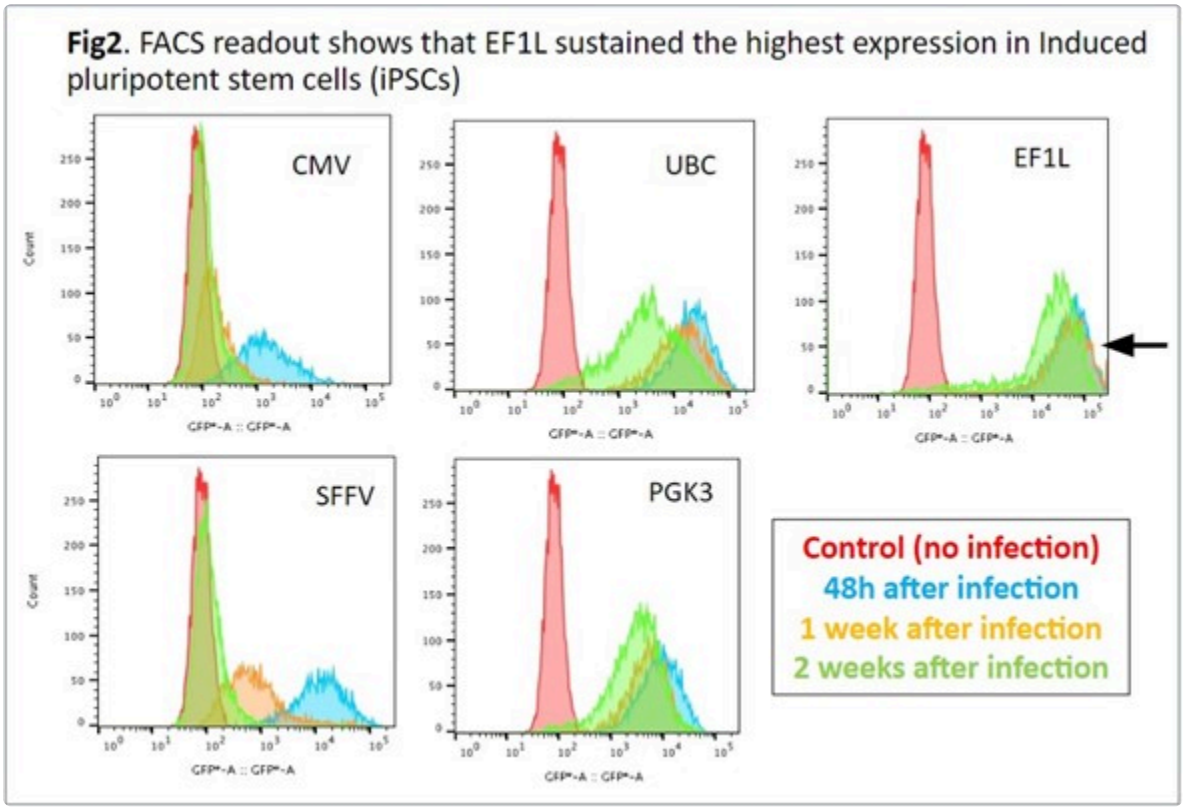
1. PromoterTest™ Custom Assay Kit

The PromoterTest™ Assay Custom Kit enables you to choose from a range of promoter constructs to easily identify the promoter that is optimally suited to express a cDNA of interest in your mammalian cell system. Each lentiviral construct is pre-packaged as ready-to-transduce lentiviral particles that can be transduced into your target cells. The lentivector carries the same green fluorescent protein (GFP) under the control of a different promoter. The intensity of the resulting fluorescence, as measured by FACS, correlates with the expression level of GFP. Higher GFP intensity indicates stronger promoter activity.

With this customizable PromoterTest™ Assay, you can measure the relative expression level between multiple promoters by assessing promoter strength (Fig.1) and sustained long-term expression (Fig.2) to find an optimal promoter that meets your expression requirements.





References and Product Citations for all Cellecta products can be found on the Cellecta website: https://www.cellecta.com/resources/publications/.

Please read the entire user manual before proceeding with your experiment. Also, please note that when working with pseudoviral particles, you should follow the recommended guidelines for working with Biosafety Level 2 (BSL-2) materials.

Click the ? Download as PDF link at the bottom of the left menu to download the PDF version of this user manual.

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2. Kit Components

The build your custom kit, you can choose from the following promoter options:

Construct	Promoter	Volume
Human EF1alpha full-length promoter	EF1L	0.5 ml
Human EF1alpha short promoter	EFS	0.5 ml
Human cytomegalovirus promoter	CMV	0.5 ml
Human cytomegalovirus promoter/enhancer	E/CMV	0.5 ml
Mouse cytomegalovirus promoter	mCMV	0.5 ml
Spleen focus forming virus promoter	SFFV	0.5 ml
Rous sarcoma virus promoter	RSV	0.5 ml
Human ubiquitin C promoter	UBC	0.5 ml
Human phosphoglycerate kinase 1 promoter	PGK	0.5 ml
Simian virus 40 promoter	SV40	0.5 ml

Other reagents, such as cell culture media appropriate for the cell lines of interest is required, and LentiTrans™ Transduction Reagent (Cat.# LTDR1) is recommended for transduction.

Generally, the user is assumed to have access to standard materials (e.g., tissue culture plates, polypropylene tubes, pipette tips), equipment (tabletop centrifuges, pipettes, scales), and common reagents (e.g., TE buffer, ethanol) and buffers used in a typical life science laboratory.

The PromoterTest Kit should be stored at -80°C until ready for use.

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3. Promoter Testing Procedure

Each prepackaged lentivector in the PromoterTest Assay has a different promoter driving expression of the same GFP reporter gene. To test the activity of each promoter in your cell line, transduce cells with each lentivector, then compare the GFP fluorescent intensity from each lentivector/promoter.

- Before starting the procedure below, plate and expand your target cells to an appropriate density to ensure they are viable and actively growing.

- The procedure below describes the overall approach to assay the promoter constructs in this Kit. For detailed guidance on the transduction procedure, refer to the **General Lentiviral Transduction Protocol** in Section 4.

Day 0: Plate and Transduce MOI Dilution Series

1. Quickly thaw the lentiviral particles for the promoter constructs in a water bath at 37°C. Transfer the thawed particles to a laminar flow hood, gently mix by rotation, inversion, or gentle vortexing, and keep on ice.

2. Suspend cells at a density of ca. 100,000 cells/ml and aliquot 1 ml of suspension into each wells of a 12-well plate. To assay all 10 constructs, aliquot 3 wells of cells for each construct, plus an additional no-virus control (25 wells total) for each replicate.

3. Transduce aliquots of 100,000 cells with 3 µl, 10 µl, 30 µl of each packaged viral construct. Do not add virus to control cells.

> **Note:** Unused reagent can be aliquoted, refrozen at -80°C, then used again for subsequent experiments. Repeated freeze/thaw will deplete viral concentrations.

Day 1: Change Medium

- Change culture medium ~24h after transduction.

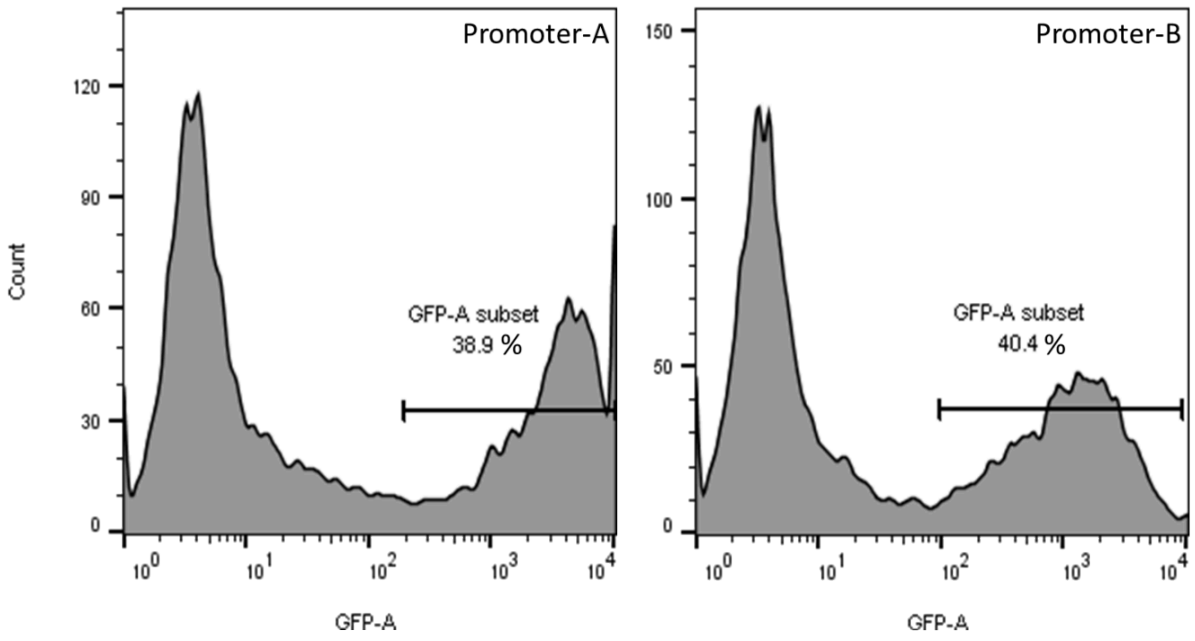
Day 3: Harvest and Analyze

1. At 48 or 72 hours after transduction, harvest the cells and check GFP fluorescent intensity by flow cytometry (488 nM excitation, 530/20 nM emission).

2. For each lentivector, select the viral transduction sample (3ul, 10ul or 30ul) which shows between 10%-40% GFP+ cells

> **Note:** If none of the cultures show between 10%-40% GFP+ cells, adjust the amount of virus as needed and redo the transductions.

3. Compare the GFP peak intensity generated by cells with each different promoter construct. The one with the highest peak intensity corresponds to the strongest GFP and, therefore, the highest promoter strength. Please see the example data below from cell populations transduced with two different promoters constructs:



Cells transduced with the Promoter-A GFP construct peak at an intensity of ~4×10^3.

Cells transduced with the Promoter-B construct peak at an intensity of ~1.5×10^3.

Based on the ratio ($4\times10^3/1.5\times10^3$), Promoter A is ~2.5 times stronger than Promoter B in these cells.

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4. General Lentiviral Transduction Protocol

This section provides the general protocol for transduction of mammalian cells with VSV-G packaged lentiviral particles. This protocol was developed and optimized using HEK293 and K-562 cells, and has been successfully used with many other common cell types. However, each cell is different and, depending on the characteristics of your specific cells, some optimization may be necessary.

Lentiviral transductions are performed by mixing cells and virus in culture media. For both adherent and suspension cells, transductions are initiated in suspension and carried out overnight. Adherent cells are allowed to adhere to substrate during transduction and are transduced at a cell density that allows for 2-3 population doublings before reaching confluence. Suspension cells are typically transduced at a higher density than standard growth density, and then they are diluted to standard growth density 18-24 hours after transduction. Do not let cells become too dense or let the medium become yellow at any point.

Before transduction, seed and expand cells from frozen stocks. Cells should be actively growing.

> ! **CAUTION:** Only open the tube containing the lentiviral particles in the laminar flow hood. Please refer to the **Safety Guidelines** and check with your institution regarding the use of lentiviruses.

Day 0—Inoculate Cells

1. Quickly thaw the lentiviral vector particles in a water bath at 37°C. Transfer the thawed particles to a laminar flow hood, gently mix by rotation, inversion, or gentle vortexing, and keep on ice. Unused viral particles can be aliquoted, refrozen at -80°C, and used again for subsequent experiments. There will be some loss of viral activity (usually 10-20%) with each refreeze.

2. Suspend sufficient cells for transduction in appropriate complete media supplemented with 1 µl/ml LentiTrans™ Transduction Reagent—if cells are sensitive to the LentiTrans Reagent, use a lower concentration or omit it. For HEK293 cells, we usually suspend at a density of 1×10^5 cells/ml in D-MEM supplemented with 10% FBS and LentiTrans Reagent. For K-562 cells, we usually suspend at a density 2×10^6 cells per ml in RPMI/10%FBS supplemented with 20mM HEPES, pH 7.4 and LentiTrans Reagent.

3. Aliquot cells into wells or plates. For small scale transductions or titering assays, cells may be plated into multiwell microtiter plates (e.g., 0.5 ml/well for 24-well plates or 1 ml/well for 12-well plates). To transduce larger numbers of cells, use larger plates and scale up the volume accordingly

4. To each plate, add an appropriate amount of lentivirus. The amount of virus will depend on your viral titer and your experiment. Refer to the Transduction Guidelines or Assay Procedures section of the product manual for application specific recommendations.

5. Close the plate and mix by gentle agitation:
    - For adherent cells, place the plate into the CO_2 incubator and grow cells under standard conditions for 16-24 hours.
    - For suspension cells only, “spinoculate” by wrapping the perimeter with parafilm, placing the plate into the centrifuge with an appropriate balance, and spin the cultures at 1,200 × g at +25°C for 2 hours. Following centrifugation, remove plate(s) from centrifuge, carefully remove parafilm, and place in incubator. After 3 hours, “feed” cells with 0.5 ml additional complete medium per well (no LentiTrans Reagent).

Day 1—Change Media

Between 16 to 24 hours post-transduction, remove media and replace with fresh complete media without LentiTrans Reagent. For suspension cells, spin down and resuspend cells in complete media at 1-5 × 10^5 cells/ml. Place in incubator and grow for an additional 24-48 hours. Avoid confluency or too high a density of cells during and after transduction. If necessary, replate.

Day 3 or 4—Harvest or Split Cells

At about 72 hours after adding virus, you may expand cells as normal or harvest cells for an assay. To continue growing cells, split the cells 1:4 to 1:8 (or as appropriate, depending on the type of cells) as the culture approaches confluence, and add complete medium. As required by your experiments add antibiotics, other factors (tetracycline), etc., and expand as normal.

NOTE: Typical doxycycline concentration for induction is 0.5 ug/ml. However, some cell lines can be more sensitive than others, so you may want to optimize the concentration.

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5. Technical Support

Email Addresses

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For the latest technical news and updates, visit Cellecta's blog at: https://cellecta.com/blogs/news

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6. Safety Guidelines

The HIV-based lentivector system is designed to maximize its biosafety features, which include:

- A deletion in the enhancer of the U3 region of 3'ΔLTR ensures self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of the target cells.
- The RSV promoter upstream of 5'LTR in the lentivector allows efficient Tat-independent production of lentiviral RNA, reducing the number of genes from HIV-1 that are used in this system.
- Number of lentiviral genes necessary for packaging, replication and transduction is reduced to three (gag, pol, rev). The corresponding proteins are expressed from different plasmids lacking packaging signals and share no significant homology to any of the expression lentivectors, pVSV-G expression vector, or any other vector to prevent generation of recombinant replication-competent virus.
- None of the HIV-1 genes (gag, pol, rev) will be present in the packaged lentiviral genome, as they are expressed from packaging plasmids lacking packaging signal—therefore, the lentiviral particles generated are replication-incompetent.
- Lentiviral particles will carry only a copy of your expression construct.

Despite the above safety features, use of HIV-based vectors falls within NIH Biosafety Level 2 criteria. For a description of laboratory biosafety level criteria, consult the Centers for Disease Control Office of Health and Safety Web site at:

> https://www.cdc.gov/biosafety/publications/bmbl5/index.htm

It is also important to check with the health and safety guidelines at your institution regarding the use of lentiviruses and follow standard microbiological practices, which include:

- Wear gloves and lab coat at all times when conducting the procedure.
- Always work with lentiviral particles in a Class II laminar flow hood.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory area are to be placed in a durable, leakproof, properly marked (biohazard, infectious waste) container and sealed for transportation from the laboratory.

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7. Contact Us

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