Data sheet

p2RVa-SEAP/ ΔNGFR Retroviral Dual Reporter

Cat. No: PC0135
Cat. No: PC0135 Plus

Description

Dual Reporter vector family provides a validated wide collection of dual reporter vectors developed for in vitro studies. This vector family is designed for high co-expression of two reporter genes drive for ubiquitous, strong and constitutive promoters.

p2RVa-SEAP/ ΔNGFR Dual Reporter is a retroviral vector that contains elongation factor 1 alpha promoter ($P_{EF1\alpha}$) to drive the expression of both secreted embryonic alkaline phosphatase (SEAP) and truncated nerve growth factor receptor (ΔNGFR). This is a self-inactivating (SIN) retroviral vector that lack viral promoter and enhancer activity in their 3' long terminal repeat (LTR).

The reporter proteins are produced in stoichiometric proportion because of the expression cassettes are based in 2A sequence. 2A-like sequence is used by several families of viruses for producing multiple polypeptides. Unlike IRES based vectors where protein expression from the insert downstream IRES is lower than of the upstream insert, 2A based vectors allow both proteins are produced in identical proportion.

2A-mediated cleavage is a universal phenomenon in all eukaryotic cells. The 2A peptides have been used successfully to generate multiple proteins from a single promoter in some biological models: plants, zebrafish, transgenic mice and human cell lines.

This dual reporter vector has reporter gene combination for different cell locations: extracellular (SEAP) and membrane (ΔNGFR). It can be used in transient transfection[1] or for produce virus to infect target cells.

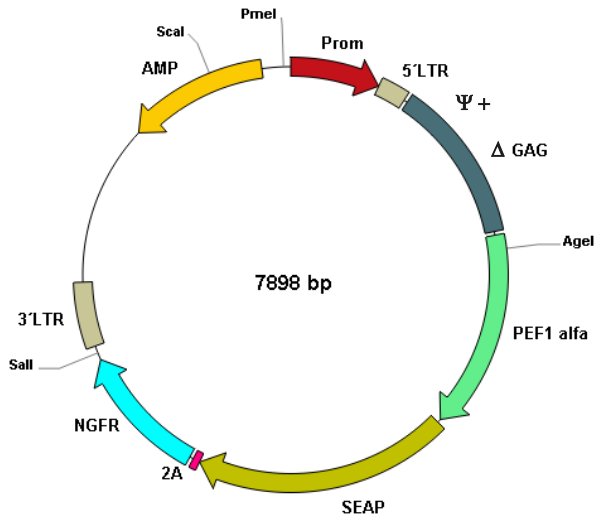
Related Products Non Provided

- SEAP Reporter Gene Assay Kit (Cat.Nº: CA040)
- NGFR Detection Kit (Cat.Nº: CA110)
- ASSEMBLE Amphotropic Viral Packaging (Cat.Nº:ME0042)
- ASSEMBLE Ecotropic Viral Packaging (Cat.Nº: ME0040)

Kit Components

Components	PC0135	PC0135 Plus
p2RVa-SEAP/ ΔNGFR (1 µg/ µL)	15 µL	15 µL
CANFAST Transfection Reagent (1 mg/ mL)	-	1 mL
ASSEMBLE Viral Packaging Vector (1 µg/ µL)▲		15 µL

▲ *ecotropic (tropism for rat or mouse cells) or amphotropic (tropism for most mammalian cells; hamster are not included).*



Unique restriction sites are shown

Biosafety Features

- Biosafety Level 2 (BL-2).
- One packaging vector
- Disposable Packaging vectors are 3´LTS SIN and packaging signal deleted (ΔΨ).
- Viruses from SIN vectors results in the transcriptional inactivation of the provirus in the infected cell.

[1] *For transfection procedure use CANFAST Transfection Reagent Manual.*

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Assay procedure

Infection Protocol

Culturing Packaging Cell Lines

We recommended use HEK-293 cell line to produce viral supernatant.

Virus Production

1. **For transfection of retroviral vector, seed your packaging cell line** at 60-80% confluence the day before transfection. The number of cells recommended for 6 well plate are 2,5-8,0x 10^5.
2. On the day of transfection, **prepare CANFAST and DNA solution**. Please use medium without serum to prepare them according to the table below.
3. Prepare the transfection mix adding **CANFAST** solution **drop to drop** into DNA solution which is gently stirring at vortex.
4. Incubate transfection mix 15-20 minutes at room temperature. Add transfection mix into each well by leaking and shake **gentle** the plate
5. Incubate for 48 – 72 hours to obtain high viral titer in the supernatant (*usually titers of 10^5 – 10^6 cfu/mL*).

   Aspire culture medium 12-24h post-transfection, and add 2ml of complete medium for each well.

Recommended Ratios CANFAST Transfection Reagent / DNA					
Tissue Culture Vessel	DNA Solution		CANFAST solution		Transfection Mix (mL)***
	DNA (µg)*	Medium without serum (µL)**	CANFAST Reagent (µL)	Medium without serum (µL)**	
6-well and 35 mm plate	2	100	6-12	100	200
60 mm plate	3-6	300	18-36	300	600
100 mm plate	8-16	800	48-96	800	1600

* *The quantity of DNA includes a half of Packaging DNA (Ref: ME0040 or ME000042) plus a half of expression vector DNA*
** *Final volume after DNA or CANFAST Reagent addition*
** *Transfection mix is the addition of volumes from 3rd and 5th columns.*

6. Collect the viral supernatant and centrifuge by low speed (about 500 x g) for 5 min at 4ºC or pass it through a 0.45 µm cellulose acetate or PES filter for removing cell debris. (Do not use a nitrocellulose filter because the virus is destroyed). Aspirate the supernatant and storage it or infect target cells.

Viral Infection of Adherent Cells

1. **Plate the cells,** the day before infection, at a cell density of 1-2x10^5 per 60 mm plate.
2. **Add viral supernatant to the culture.** Use as much virus-containing medium as possible or if you know the viral titer, use the volume which you need. If you need improve yours results, you can do multiple rounds of infection. Allow cells to rest for 12-24 h between each infection.
3. **Add polybrene** to a final concentration of 8 µg/mL. Polybrene range is 2 – 12 µg/mL. Could be necessary optimized this parameter.
4. **Replace medium** with fresh medium after 24h of infection. You can replace medium after 4 – 6h after infection if your target cells require it.
5. After 48- 72hr infection you can analyse the results of infection process.

Viral Infection of Suspension Cells (Protocol for 6-well plate)

1. **Collect the cells** by centrifugation (250g for 5 min) and **resuspend** 1x10^6 cells in 1 mL of the filtered viral supernatant.
2. **Add polybrene** to a final concentration of 8 µg/mL and dispense the mix into a well of the 6-well plate.
3. **Centrifuge the plate** at 1000g, 1 h 32ºC (*32ºC increases viral half-life*).
4. **Incubate** the plate for 4-6 h.
5. **Replace medium** with 2mL of fresh medium and **incubate** the cells at 37ºC, 5% CO_2.
6. After 48- 72hr infection you can the results of infection process.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.canvaxbiotech.com for Material Safety Data Sheet of the product.

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