Data sheet

p2V-SEAP/ ΔNGFR-IIa Dual Reporter

Cat. No: PC0114
Cat. No: PC0114 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.

p2V-SEAP/ ΔNGFR-IIa Dual Reporter vector 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).

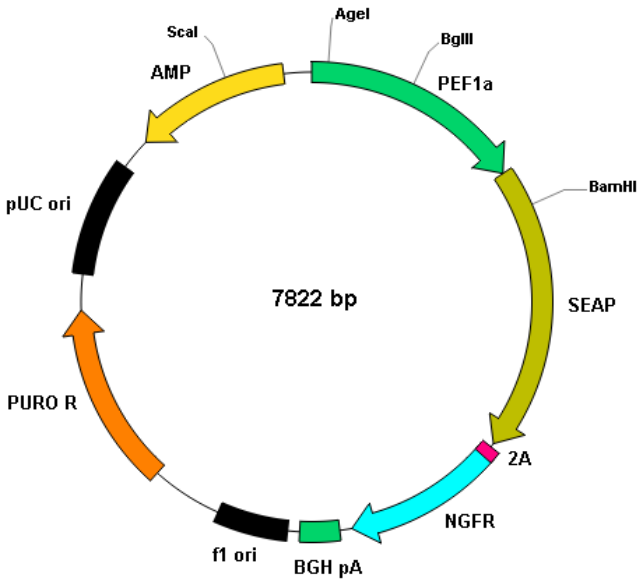
The reporter proteins are produced in stoichiometric proportion because of the expression cassettes are based in 2A sequence. 2A-like sequence is used by several families of viruses for producing multiple polypeptides. Unlike IRES based vectors where protein expression from the insert downstream IRES 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 can be used in transient or in stable transfection and it is available with puromycin mammalian resistance marker and with some dual reporter gene combination for different cell locations: extracellular (SEAP) and membrane (ΔNGFR).

Kit Components

Components	PC0114	PC0114 Plus
p2V-SEAP/ ΔNGFR-IIa (1 µg/ µL)	15 µL	15 µL
CANFAST Transfection Reagent (1 mg/ mL)	-	1 mL



Unique restriction sites are shown

Related Products Non Provided

- SEAP Reporter Gene Assay Kit (Cat.Nº: CA040).
- NGFR Detection Kit (Cat.Nº: CA110).

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Assay procedure

Transfection Protocol (stable or transient)

1. **For adherent cells, seed the cells** 18-24 hours before transfection to obtain 60-80% confluence the day of transfection, according the next table.

   For suspension cells, seed the cells the day of transfection. Seed the cells to obtain 60-80% confluence according to the table below. The number of cells to seed depends on cell growth.

Recommended Number of Cells to seed for CANFAST Transfection			
Tissue Culture Vessel	**Growth Area (mm^2)**	**Cell number/ well**	**Final volume/ well (mL)**
Adherent Cells to Seed			
24 well plate	200	$6{,}0{\cdot}10^4$-$2{,}0{\cdot}10^5$	0,5
6 well plate	962	2,5-$8{,}0{\cdot}10^5$	2
Suspension Cells to Seed			
24 well plate	200	$2{,}0{\cdot}10^4$-$1{,}0{\cdot}10^5$	0,5
6 well plate	962	1,0-$5{,}0{\cdot}10^5$	2

   On the day of transfection, it is not necessary to change the medium

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.

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)***	
96 well plate	0,15	7,5	0,4-1	7,5	15
48 well plate	0,3	15	1-1,8	15	30
24 well plate	0,6	30	2-4	30	60
12 well plate	1	50	2-6	50	100
6 well plate	1-2	100	6-12	100	200
35 mm plate	1-2	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

* *Final volume after DNA or CANFAST Reagent addition*
** *Transfection mix is the addition of volumes from 3rd and 5th columns.*

4. Incubate transfection mix 15-20 minutes at room temperature.
5. **For transfection**, add transfection mix into each well by leaking. Gentle shake the plate and incubate it 24 – 72 hours. Some cell lines are more sensitive and require change the culture medium 1 – 16 hours after adding the transfection mixture to avoid toxicity.

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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