

Data sheet

pOnebyOne™-III-Lentiviral

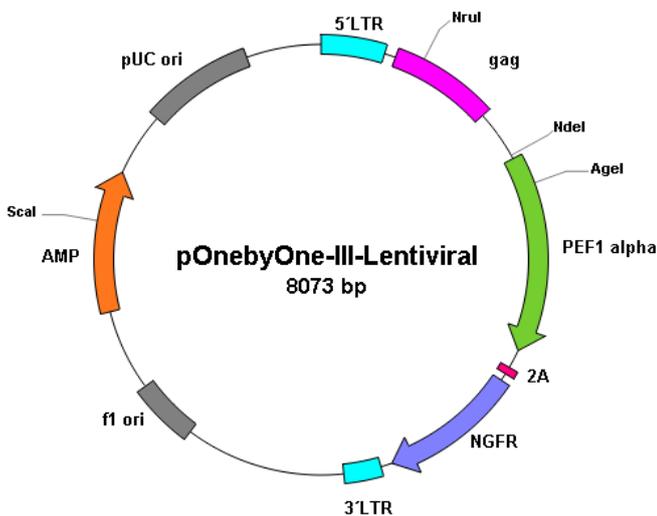
Cat. No: ME0019 (20 reactions)

Description

pOnebyOne™-III-Lentiviral mammalian expression vector contains an expression cassette based in 2A sequence. 2A sequence allows multiple proteins to be encoded as polyproteins and unlike IRES based vectors both proteins are produced in stoichiometric proportion.

The expression cassette of pOnebyOne™-III-Lentiviral contains human elongation factor 1 alpha promoter that precedes 2A sequence in frame with truncated nerve growth factor receptor (Δ NGFR). Δ NGFR is a complete solution to selected positive clones. They could be visualized by cytometry using specific antibody labelled with FITC or similar and also, they could be enriched from negative clones with magnetic beads bearing anti- Δ NGFR antibody.

pOnebyOne™ vector family includes **ready to use** vectors for an easy and highly efficient cloning procedure. The vectors are linearized, just for join with your PCR amplified with the recommended primers. Experimental background is less than 2%.



Unique restriction sites are shown.

Kit Components

ME0019

pOnebyOne™-III-Lentiviral (50 ng/ μ L)*	20 μ L
10X Glue Enzyme Buffer	50 μ L
Glue Enzyme (10 UI/ μ L)	40 μ L
Control Insert DNA (30 ng/ μ L)	10 μ L
pOnebyOne™-III-Lentiviral Control**	5 μ L

* Linearized vector

** Circular vector. Empty vector for transfection and expression control

Biosafety Features

- ✓ Biosafety Level 2 (BL-2).
- ✓ Required three packaging vectors carrying rev, gag/pol and env genes.
- ✓ 3'LTR HIV deleted: SIN vectors result in the transcriptional inactivation of the provirus in the infected cell.
- ✓ Packaging signal deleted ($\Delta\Psi$ -GAG).
- ✓ Utilize a chimeric 5'LTR promoter to ensure transcription in absence of Tat.

Features

Promoter RSV	1-230
5'LTR	231-410
Ψ +/ Δ GAG packaging signal	463-1115
Human elongation factor 1 alpha promoter	1812-3002
2A from equine rhinitis A virus	3011-3073
Δ NGFR	3080-3916
3'LTR SIN (self inactivated deletion)	4593-4828
Ampicillin resistance gene (ORF)	5988-6845
pUC origin	6993-7666

(Continued on reverse side)

Canvax Biotech, S.L. C/ Astronoma Cecilia Payne. Edif. Orion. 14014 Cordoba, Spain.

☎ : +34 957 348 066

☎ : +34 957 346 217

✉ : info@canvaxbiotech.com



Assay procedure

Cloning

1. Check your PCR has been amplified with the correct primers (*see Manual of pOnebyOne™ Lentiviral Vectors*).
2. Spin pOnebyOne™ Lentiviral vector to collect content at the bottom of the tubes.
3. **On ice**, set up reaction as described below. If you thawed all kits components out of ice, you must pre-chill all them before use during 10 minutes.

Match Reaction	Cloning Reaction	Control Reaction	Background Reaction
pOnebyOne™ vector (50 ng/μL)	1 μL	1 μL	1 μL
10x Glue-Enzyme Buffer	1.5 μL	1.5 μL	1.5 μL
PCR Product**	X μL	-	-
Control Insert DNA	-	2 μL	-
Water (Molecular Biology grade)	up 13 μL	up 13 μL	up 13 μL

** Relation vector: insert 1:5 is recommended

4. Mix the reactions by pipetting.
5. Incubate 10 minutes on ice.
6. Add 2 μL Glue-Enzyme (10 U/μL) to each tube, mix gently and incubate 45 minutes on ice.

Transformation

1. Centrifuge the tubes containing the reactions to collect content at the bottom of the tube. Add 15 μL of each reaction to a sterile 1.5 mL microcentrifuge tube on ice. Set up another tube on ice with 50 pg uncut plasmid (*not supplied*) for determination of the transformation efficiency of the competent cells.
2. Remove a tube of frozen Competent Cells (*not supplied*) from storage at -80°C and place in an ice bath until just thawed (about 10 to 15 minutes). Mix the cells by **gently** flicking the tube with your fingertips.
3. **Carefully** transfer 50 μL of cells into each tube prepared in **Step 1**.
4. **Gently** flick the tubes to mix and place them on ice for 30 minutes.
5. Heat-shock the cells for exactly 45 seconds in a water bath at exactly 42°C (**Do not shake**).
6. Immediately return the tubes to ice for 2 minutes and plate all transformation mix onto pre-warmed LB ampicillin plates
7. Incubate the plates overnight (12–16 hours) at 37°C.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. Please refer to www.canvaxbiotech.com for Material Safety Data Sheet of the product.