

Data sheet

pOnebyOne®-III-Retroviral

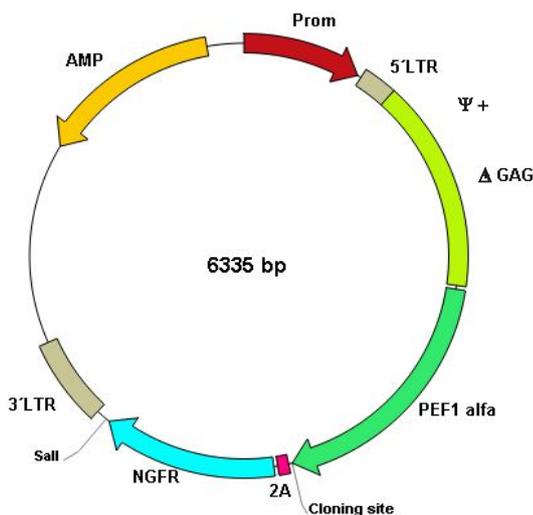
Cat. No: ME0015 (20 reactions)

Description

pOnebyOne®-III-Retroviral 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-Retroviral 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	ME0015
pOnebyOne®-III-Retroviral (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-Retroviral Control**	5 μ L

* Linearized vector

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

Biosafety Features

- ✓ Biosafety Level 2 (BL-2).
- ✓ One packaging vector
- ✓ Viruses from SIN vectors result in the transcriptional inactivation of the provirus in the infected cell.
- ✓ Disposable Packaging vectors are 3'LTR SIN and packaging signal deleted (Δ Ψ -GAG).

Features	
Prom (Citomegalovirus promoter)	8-567
5'LTR	574-748
Ψ +/ Δ GAG packaging signal	749-1747
Human elongation factor 1alpha promoter	1755-2956
2A from equine rhinitis A virus	2966-3028
Δ NGFR	3035-3870
3'LTR SIN (self-inactivated deletion)	3926-4352
Ampicillin resistance gene (ORF) (Complementary strand)	5306-6162

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Assay procedure

Cloning

1. Check your PCR has been amplified with the correct primers (*see Manual of pOnebyOne*).
2. Spin pOnebyOne® 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 (<i>Molecular Biology grade</i>)	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 nor heat shock more than 45 seconds**).
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. The product was not tested for use in diagnostics or for drug development, nor it is suitable for administration to humans or animals. Please refer to www.canvaxbiotech.com for Material Safety Data Sheet of the product.