Data sheet

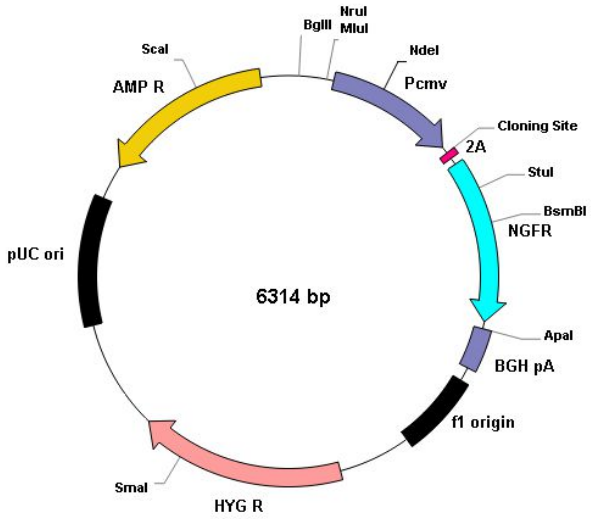
pOnebyOne®-I-Hygro

Cat. No: ME0001-H (20 reactions)

Description

pOnebyOne®-I-Hygro 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®-I-Hygro contains the cytomegalovirus early promoter that precedes 2A sequence in frame with truncated nerve growth factor receptor (ΔNGFR). ΔNGFR 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-ΔNGFR antibody. Stable mammalian cells could be selected by hygromycin resistance.



Unique restriction sites are shown

Kit Components

Components	ME0001-H
pOnebyOne®-I-Hygro (50 ng/ µL)*	20 µL
10X Glue Enzyme Buffer	50 µL
Glue Enzyme (10 UI/ µL)	40 µL
Control Insert DNA (30 ng/ µL)	10 µL
pOnebyOne®-I-Hygro Control**	5 µL

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

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

Features	
Cytomegalovirus promoter	*232-887*
Cloning site	*915*
2A from equine rhinitis A virus	*919-981*
ΔNGFR	*988-1823*
BGH polyadenylation sequence	*1858-2082*
f1 origin	*2138-2556*
Hygromycin resistance gene (ORF)	*2909-3942*
pUC origin	*4503-5173*
Ampicillin resistance gene (ORF) (complementary strand)	*5321-6178*

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Assay procedure

Cloning

1. Ckeck 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	1µL	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 -80ºC 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.

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