Cat. # 6160

For Research Use

TaKaRa

Retrovirus Packaging Kit Eco

Product Manual

v202103Da

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Precautions for use of this product

- Please follow the guideline for experiments using recombinant DNA issued by the relevant authorities and the safety committee of your organization or your country in using this product.
- The use of this product is limited only for research purposes. It must not be used for clinical purposes or for *in vitro* diagnosis.
- Individual license agreement must be concluded when this product is used for industrial purposes.
- Recombinant retrovirus solution may include virus with unknown hazardous gene. Please use a safety cabinet and gloves to prevent inhalation or adhesion of the virus.
- Basic techniques of genetic engineering and cell cultivation are needed for the use of this product.
- **The user is strongly advised not to generate recombinant retrovirus capable of expressing known oncogenes and any genes known to be hazardous to the mammals.**
- **Takara is not liable for any accidents or damages caused by the use of this product.**

I. Description

This kit is designed to obtain transiently high-titer recombinant retrovirus particles by co-transfection of retrovirus vector plasmid with target gene and two unique vectors for virus packaging, using calcium phosphate method.
This kit contains a *gag-pol* expression vector and an ecotropic *env* expression vector. The recombinant virus obtained using this kit are able to infect most mouse and rat cells.
The packaging vectors have retrovirus structural genes (*gag-pol* and *env* gene) which are necessary to construction/replication of virus particles, but don't have ψ (packaging signal) and LTR sequence.
By co-transfecting the recombinant retrovirus vector plasmid which has ψ and LTR with these packaging vectors to 293 cells or 293T cells using transfection reagents supplied in this kit,transiently high-titer recombinant retrovirus particles are obtained after 48 hours.
These packaging vectors are highly purified and ready to use for transfection.
There is little possibility of getting the replication competent retrovirus, because these packaging vectors don't have any DNA sequence from retrovirus except *gag-pol* and *env* gene.
The kit is compatible with all retroviral transfer vectors sold by Takara Bio, including MMLV- and MSCV-based retroviruses.

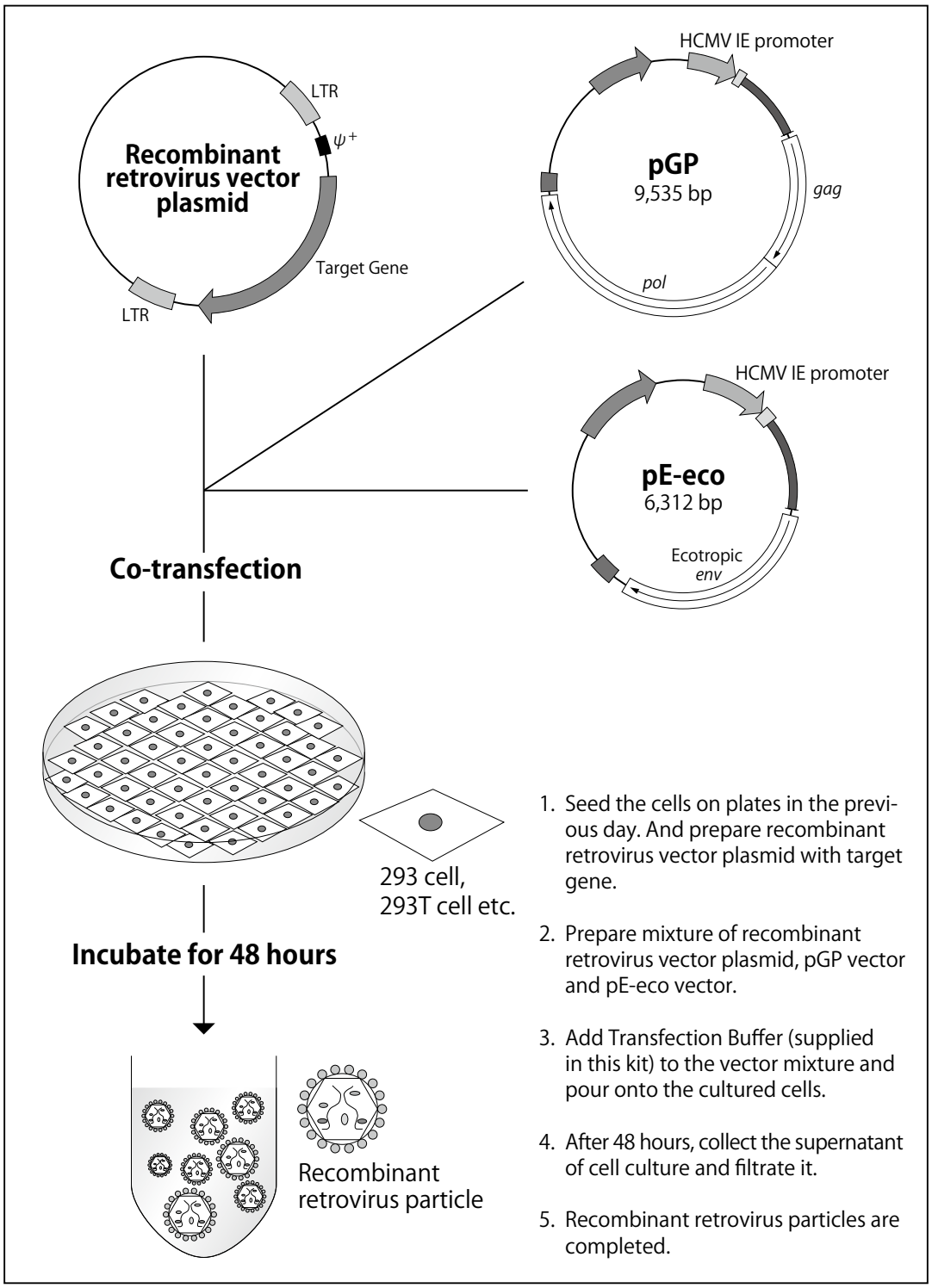

Figure 1. Principle of the generation of recombinant retroviruses

II. Components (for 10 reactions)

This kit contains two packaging vectors and transfection reagents.

1.	pGP Vector (1 μg/μl)	50 μl	
2.	pE-eco Vector (1 μg/μl)	50 μl	
3.	Transfection Buffer	500 μl	x 10
4.	2 M $CaCl_2$	620 μl	
5.	25 mM Chloroquine	40 μl	

III. Storage

-20℃
(Transfection Buffer, 2 M $CaCl_2$ and 25 mM Chloroquine should be stored at 4℃ after thawing them.)

Note : The kit is stable for 2 years from date of receipt when unused and stored properly at -20℃.
Once opened or thawed, store at each component under proper storage conditions, and use sooner avoiding contamination.

IV. Material Required but not Provided

【 Instruments and equipments 】

- Safety cabinet
- Microscope for cell observation
- Humidified CO_2 incubator
- -80℃ freezer
- Sterilized pipette tips with filters
- Sterilized 5.0 ml round-bottomed tubes (polystylene)
- Sterilized 2.0 ml tubes (for storage of viruses)
- Sterilized 0.45 μm filters (low adsorption)
- Gelatin or collagen coated dishes (φ 6 cm)
- Sterilized pipettes
- Electoric pipetter

【 Reagents 】

- Recombinant retrovirus vector plasmid (highly purified)
- Fetal Bovine Serum (FBS)
- Trypsin - EDTA solution
- 293 cells, 293T cells [2] etc.
- Dulbecco's Modified Eagle's Medium (DMEM) with Glucose (4.5 g/L) and L-Glutamine (584 mg/L)
- Penicillin/Streptomycin
- Sterile purified water

V. Protocol

1. Preparation (the previous day)

Seed 2 - 3 x 10^6 of 293 cells or 293T* cells into one φ 6 cm gelatin or collagen coated dish.

* 293T cells [2], which were made by introducing SV40 T antigen gene into 293 cells, are useful for transient transfection and can produce higher titer virus than 293 cells.

2. Transfection (1st day)

Bring the transfection reagents and sterile purified water to room temperature. Operate the following steps preventing any contamination.

1. Prepare the following vector mixture in a sterilized 5.0 ml round-bottomed tube (polystylene).

Recombinant retrovirus vector	10 μg
pGP Vector (1 μg/μl)	5 μl
pE-eco Vector (1 μg/μl)	5 μl
2 M $CaCl_2$	62 μl
Sterile purified water	
	up to 500 μl

2. Change the culture medium to 3 ml of DMEM with 10% FBS and 25 μM chloroquine.
3. Form calcium phosphate precipitation and transfect to cells.
   1) Suck up 500 μl of Transfection Buffer using electoric pipetter.
   2) Add the buffer gently to the vector mixture with shaking the tube.
   3) Bubble the solution immediately by using exhaust of pipetter for 10 - 20 sec to accelerate the formation of calcium phosphate precipitation.
   4) Within 1 - 2 min, drop the solution onto the cell dish evenly and rock the dish gently several times.
   5) Incubate for 7 - 11 hours in 5% CO_2 incubator at 37℃.
      When calcium phosphate precipitation is formed successfully,it looks like powder-snow on the surface of cells under a microscope.
   6) Remove 3 ml of the medium from the dish, and then add 4 ml of fresh DMEM with 10% FBS. (Complete removal of culture medium may reduce the transfection efficiency, so it is recommended to leave 1 ml of medium in the dish.)

3. Exchange of medium (2nd day)

After 24 hours of transfection, change the culture medium again to 4 ml of DMEM with 10% FBS.

4. Virus recovery (3rd day)

After 48 hours of transfection, collect the culture medium and filtrate with 0.45 μm sterilized filter. For storageof virus solution, it must be dispensed each in a small amount and stored at -80℃.
Avoid repeating freeze-thaw cycles of the virus solution.

Note : The titer of the recombinant virus prepared using this kit is varied by size of inserted target gene in retrovirus vector, efficiency of transfection,etc. Generally, the titer is 10^5 - 10^7 infectious units/ml.

[Titer (cfu) measurement of virus prepared using pDON-AI-2 Neo DNA as retrovirus vector]

A. Previous day: Preparation
Seed 5 x 10^4/well of the cells (NIH/3T3 etc.) for titer assay in 6 well plate.

B. 1st day : Infection
1. Change culture medium to 900 μl of serum-containing medium with 9 μg/ml of polybrene.
2. Dilute the virus solution with serum-containing medium to 10^{-1} - 10^{-5} conc.
3. Add 100 μl of diluted virus solution to each well.
(The final conc. of polybrene is 8 μg/ml.)
4. Incubate for 4 - 6 hours in 5% CO_2 at 37℃. And then add 1ml of serum-containing medium to each well.

C. 2nd day : Medium exchange
Change medium to serum-containing medium with 400 - 800 μg/ml of G418. Then change the medium every 3 - 4 days.

D. about 2 weeks later :
1. Stain the plate with methylene-blue solution or by Giemsa method, and count the stained colonies.
2. The titer unit of the virus solution (cfu/ml) is calculated by multipling the number of colonies and dilution times of virus solution.

Note : In the case using MLV-based retrovirus vector, it is possible to assay more rapidly the RNA titer (copies/ml) with Retrovirus Titer Set (for Real Time PCR) (Cat. #6166) by real-time PCR method.

VI. References

1) Pear W S, Nolan G P, Scott M L, and Baitimore D. *Proc Natl Acad Sci USA.* (1993) **90**: 8392-8396.
2) DuBridge R B, Tang P, Hsia H C, Leong P M, Miller J H, Calos M P. *Mol Cell Biol*. (1987) **7**: 379-387.

VII. Related Products

pDON-5 Neo DNA (Cat. #3657)
pDON-5 DNA (Cat. #3658)
pDON-AI-2 Neo DNA (Cat. #3653)
pDON-AI-2 DNA (Cat. #3654)
pMEI-5 Neo DNA (Cat. #3655)
pMEI-5 DNA (Cat. #3656)
RetroNectin® Recombinant Human Fibronectin Fragment (Cat. #T100A/B)
RetroNectin® Precoated Dish (Cat. #T110A)
Retrovirus Titer Set (for Real Time PCR) (Cat. #6166)