Cat. # 6161

For Research Use

TaKaRa

Retrovirus Packaging Kit Ampho

Product Manual

v202002Da

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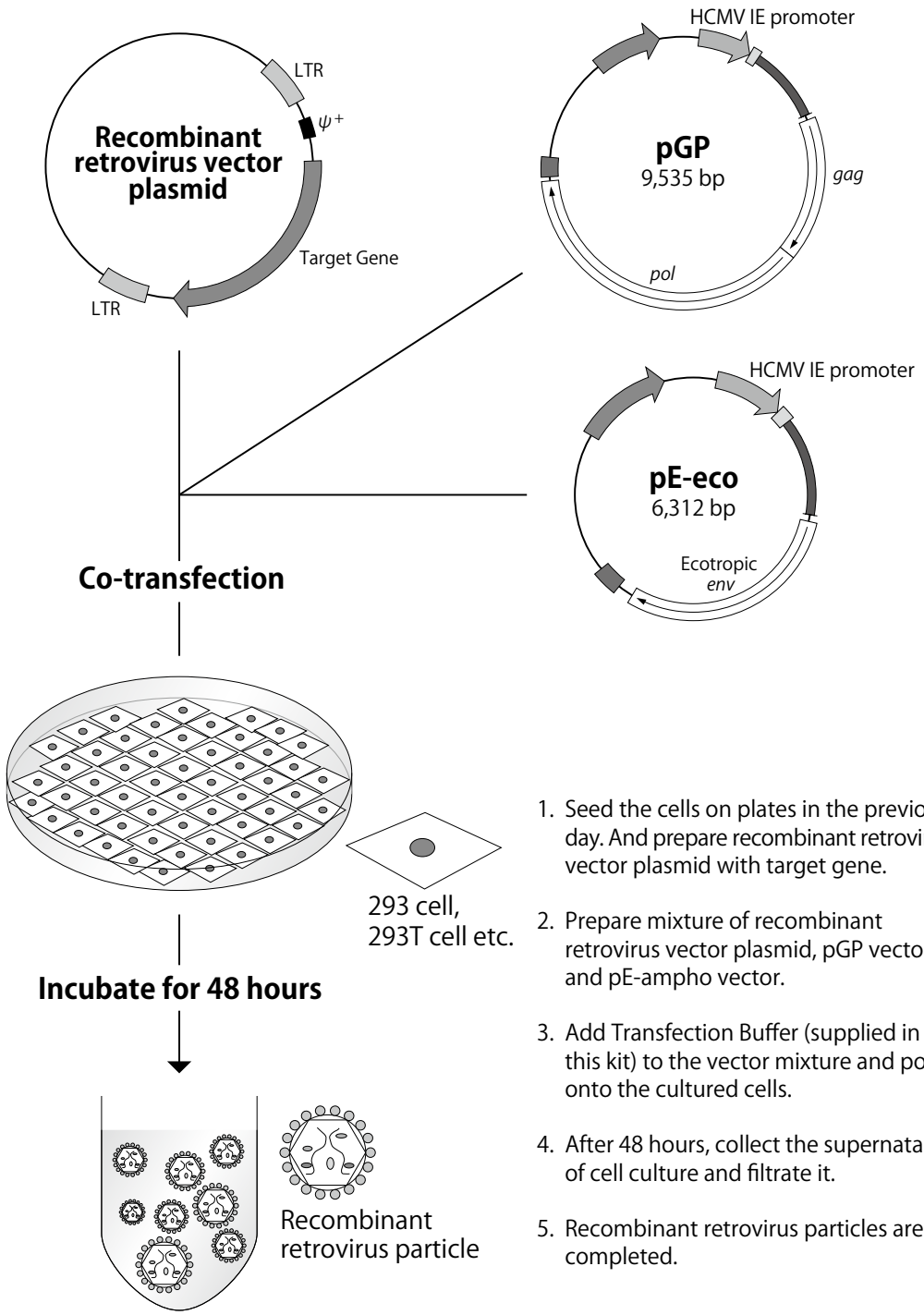
Precautions for the use of this product

- 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 Bio is not liable for any accidents or damages caused by the use of this product.

I. Description

The Retrovirus Packaging Kit Ampho is designed to obtain transient high-titer recombinant retrovirus particles by co-transfection of retrovirus vector plasmid with target gene and two unique vectors for packaging, using calcium phosphate method.
This kit contains a *gag-pol* expression vector , pGP Vector, and an amphotropic *env* expression vector , pE-ampho Vector, as packaging vectors. The recombinant virus obtained using this kit are able to infect most mammalian 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 HEK293 or HEK293T cells high-titer recombinant retrovirus particles are obtained after 48 hours.
The packaging vectors are highly purified and ready to use for transfection.
There is little possibility of getting replication competent retrovirus, because these packaging vectors don't have any DNA sequence from retrovirus except for *gag-pol* and *env* gene.

II. Principle



1. Seed the cells on plates in the previous day. And prepare recombinant retrovirus vector plasmid with target gene.
2. Prepare mixture of recombinant retrovirus vector plasmid, pGP vector and pE-ampho vector.
3. Add Transfection Buffer (supplied in this kit) to the vector mixture and pour onto the cultured cells.
4. After 48 hours, collect the supernatant of cell culture and filtrate it.
5. Recombinant retrovirus particles are completed.

Figure 1. Principles of the generation of recombinant retroviruses

III. Components (for 10 reactions)

1. pGP Vector (1 μg/μl)	50 μl
2. pE-ampho 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

IV. Storage -20℃

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

Note : 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.

V. Materials Required but not Provided

1. 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
- Sterilie purified water

* 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. Materials

- 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 (φ6 cm)
- Sterilized pipettes
- Electoric pipetter

VI. Protocol

A. Preparation (previous day)

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

B. Transfection (1st day)

Bring the Transfection Buffer, 2 M CaCl2, and sterile purified water to room temperature. Add one thousandth volume of 25 μM Chloroquine into DMEM medium with 10% FBS, and incubate at 37C.
Prepare 3 ml of the medium/6 cm dish. Operate the following steps avoiding any contamination.

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

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

2. Change the culture medium to DMEM with 10% FBS and 25 μM Chloroquine.

3. Prepare calcium phosphate precipitation and transfect to cells.
   1) Aspirate 500 μl of Transfection Buffer using electoric pipetter.
   2) Add the Transfection buffer gently to the vector mixture from step 1 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.

4. 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 during the medium change.

C. Exchange of medium (2nd day)

At about 24 hours after transfection, change to fresh DMEM with 10% FBS (4 ml).

D. Virus recovery (3rd day)

At about 48 hours after transfection, collect the culture medium and filtrate the medium with 0.45 μm sterilized filter. For storage, the virus solution must be dispensed in a small amount and stored at -80℃.
Avoid the stored virus solution to be repeated freeze-thaw cycles.

Note :

The titer of the recombinant virus obtained with this kit is dependent on genomic DNA size of retrovirus and efficiency of transfection. The virus titer obtained usually 10^5 - 10^7 infectious U/ml.

VII. Appendix

【Titer assay of virus prepared using pDON-AI-2 Neo DNA as retrovirus vector】

[Previous day : Preparation]

Seed at 5 x 10^4 cells/well the cells for titer assay (NIH/3T3 etc.) in 6 well plate.

[1st day : Infection]

1. Change the 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}.
3. Add 100 μl of the 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.

[2nd day: Medium exchange]

Change medium to serum-containings medium with 400 - 800 μg/ml of G418. Then change the medium every 3 - 4 days.

[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 multiplying the number of colonies and dilution times of virus solution.

＊ In case using retrovirus vector derived from MLV, Retrovirus Titer Set (for Real Time PCR) (Cat. #6166) is useful for rapidly measuring virus RNA titer by real-time RT-PCR

VIII. References

1) Pear W S, Nolan G P, Scott M L, and Baitimore D.
Proc Natl Acad Sci. (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.

IX. 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® Dish (RetroNectin Pre-coated Dish, 35 mm φ) (Cat. #T110A)

pSINsi-hH1 DNA (Cat. #3660)*
pSINsi-hU6 DNA (Cat. #3661)*
pSINsi-mU6 DNA (Cat. #3662)*

Retrovirus Constructive Cell (G3T-hi) (Cat. #6163)*
Retrovirus Constructive System Eco/Ampho (Cat. #6164/6165)*
Retrovirus Titer Set (for Real Time PCR) (Cat. #6166)

Human iPS Cell Generation™ All-in-One Vector (Cat. #3671)*
Human iPS Cell Generation™ Vector Set (Cat. #3670)*

* Not available in all geographic locations. Check for availability in your area.

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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