Product Information

PEG Virus Precipitation Kit

I. Kit Contents:

Components	K2190-50 50 preparations	K2190-200 200 preparations	Part Number
PEG Solution (5X)	125 ml	4 X 125 ml	K2190-C-1
Virus Re-suspension Solution (1X)	10 ml	4 X 10 ml	K2190-C-2

II. Introduction:

The PEG Virus Precipitation Kit offers a fast and convenient way for concentrating virus without ultra-centrifugation. The kit can be utilized in both small lab sample and large scale virus preparation. It is suitable for concentrating retroviruses, lentiviruses and baculoviruses etc. with high yield and high viral titer. Virus can be concentrated more than 100 folds using non-toxic reagents in this kit. An optimized Virus Re-suspension Solution is provided to maximize viral recovery by 40 - 100% depending on the virus type and sources. The concentrated virus has board applications in viral DNA, RNA purification or infection etc.

III. Reagent Storage Conditions:

The solutions are ready to use and stable for 12 months at +4℃ or at -20℃ for long term storage.

IV. Virus Precipitation Protocol:

The following protocol is designed for 10 ml virus solution. You can proportionally adjust the volumes according to your sample volume. The kit is also available for larger volume samples. Please inquire.

1. Infect cells or transfect and allow maximum virus accumulation.

2. For mammalian cell - virus or insect - baculovirus, centrifuge culture at 3,200 x g for 15 min at 4℃ to remove cells debris. For bacterial phage, centrifuge at 16,000 x g for 15 min at 4℃ to remove cells debris.

3. Collect supernatant and add 2.5 ml of PEG solution A to 10 ml of virus supernatant. Refrigerate overnight (stable up to 2 days at 4℃).

4. Centrifuge at 3,200 x g for 30 min at 4℃, carefully remove supernatant by aspiration. The beige or white pellet is the virus.

5. Suspend the virus pellet in

Notes:

1) For high titer virus preparation, the resuspension volume should be limited to about three times the volume of the white pellet, usually 1/10 to 1/100 volume of original sample. If insoluble material is present in the viral suspension, it can be removed by centrifuge at 3,200 x g for 15 min at 4℃.

2) Avoid freeze/thaw cycles to maximize virus recovery.

3) Trace amounts of PEG in the virus suspension will not affect the use of the concentrated virus. In some cases, PEG may increase virus infection efficiency. However, if it is desired, the trace amount of PEG can be removed by the following procedure:

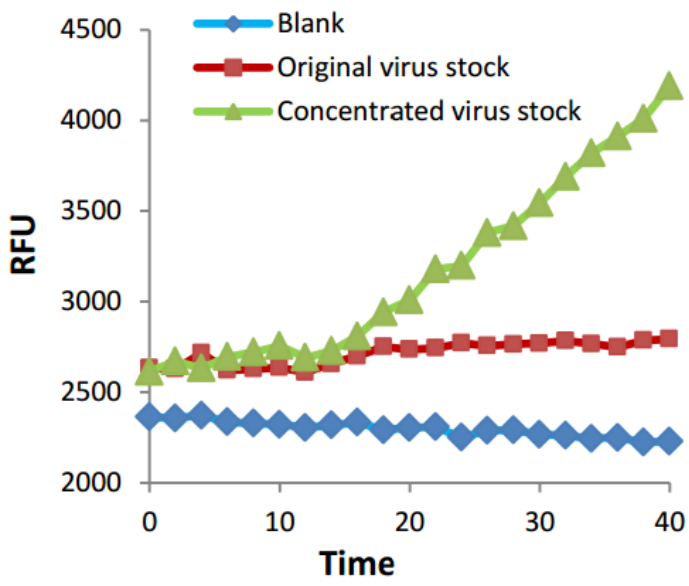
i) Add 1 volume of solution containing 4 M KCl and 50 mM Tris-HCl, pH7.2 (not provided) to 3 volumes of the concentrated virus suspension.

ii) Alternatively, add solid KCl into the virus suspension to a final concentration of 1 M.

iii) Let stand on ice for 15 - 30 min.

iv) Spin at 12,000 x g for 10 min at 4℃ to remove the precipitate.

v) Carefully collect the virus supernatant. Aliquot and store at -70℃ for future use.



IV. Frequently Asked Questions

1. If PEG is removed using the KCl protocol, how much KCl is present in the virus stock and whether this can affect cells being exposed to the virus during downstream experiments?

We think that the KCl is mostly going to be in the virus supernatant. If having too much potassium is an issue for your experiments, you could use a desalting column or dialysis to remove the potassium from the virus stock. Checking the pH of the virus solution will also give you some idea about the potassium content. We have not required desalting when we check the functioning of this kit. So there might be some optimization required to make sure your virus recovery is good after desalting/dialysis.

2. Is it necessary to incubate overnight?

Since our goal in designing this kit is to get the maximum possible viral recovery, we recommend the overnight incubation. Typically 10-12 hours incubation time is required for 95% or greater recovery. You can surely try to reduce the time, of course with reduced viral recovery, but we have not tested this.

3. Does the virus resuspension solution contain any salts? Salts would interfere in our EM analysis.

There are salts and some glycerol in the resuspension solution.The best way to ensure there are no salts in the viral prep at all is to resuspended the viral pellet in any solution of choice appropriate for the downstream application.

4. What types of viruses can this kit be used with?

The kit can be used to concentrate retroviruses, baculoviruses, lentiviruses, and phages over 100 fold.

5. Is lysis of the host cell necessary to collect virus? What about plant viruses?

Typically, virus is released into the medium and hence there is no cell-lysis required to collect virus. We have not tested this kit with plant viruses but the precipitation method should work by the same principle as all other classes of virus.

6. Can this PEG virus precipitation kit can be used with samples that have been flocculated first?

We have not tested this. But typically Flocculation is a method used to concentrate the virus. A second round could be done using our PEG precipitation kit. We have not received customer feedback about a similar situation. For many virus types precipitation is far more effective than metal based flocculation.

7. What is the advantage of using this kit over the PEG solution alone?

PEG solution alone will not be as efficient in viral recovery and storage for later use. An optimized Virus Resuspension Solution maximizes viral recovery by 40 – 100 % depending on the virus type.

8. If I have a sample containing multiple viruses, will this kit be able to precipitate all of these viruses in the sample? Will the percent recoveries be similar or will the kit favor one type of virus over another?

As long as the relative amounts of all the viruses in solution are similar, the precipitation should recover similar proportions. In other words, if there is 30% virus A, 30% virus B and 30% virus C, the relative recovery should be comparable between the viruses. We have not tested with a complex combination of viruses in the same solution.

9. Can this kit be used for Adenoviruses?

We have not tested this kit with adenoviruses per se. But since lentiviral and adenoviral particles are between 80 - 100 nm in size, they should be comparably precipitated by this kit.

10. What is the shelf-life of this kit?

Shelf-life is 1 year when stored at -20℃.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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