A&A BIOTECHNOLOGY
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Manual

Virus Mini AX Transfect

Increased efficiency kit for viral DNA purification for transfection.
Procedure with DNA precipitation.

catalog #	size
060-50T	50 isolations

For research use only.

Guarantee
A&A Biotechnology provides a guarantee on this product.
The company does not guarantee the correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

Table of Contents

Contents

component	060-50T	storage
Virus Mini AX columns	50 pcs	2–8 °C
2 ml tubes	50 pcs	15–25 °C
O purification solution	2 x 1,1 ml	-20 °C
L1.4E lysis solution	50 ml	15–25 °C
K1 equilibrating solution	45 ml	15–25 °C
K2 wash solution	170 ml	15–25 °C
K3 elution solution	60 ml	15–25 °C
PM precipitation mix	45 ml	15–25 °C
TE buffer	5 ml	15–25 °C
Proteinase K	1,1 ml	2–8 °C

The binding capacity of the minicolumn is 15 µg.

Additional equipment and reagents

Necessary

- 2 ml Eppendorf tubes
- 15 ml Falcon tubes
- 70% ethanol
- Incubator or thermoblock set to 37 °C, 50 °C
- Microcentrifuge

Optional

- Sterile water (cat.# 003-075, 003-25)

Protocol

1. Transfer **600-900 µl** of **virion solution** (viral DNA) suspended in low-salt buffer (e.g. TE buffer) to 2 ml Eppendorf tube (not included).

2. Add **40 µl** of **O** purification solution.

3. Mix the sample by inverting the tube and incubate for **30 min** at **37 °C**.

4. Add:
1 volume of **L1.4E** lysis solution - proportion 1:1, e.g. add 1 ml of L1.4E solution to 1 ml of virion solution
20 µl of **proteinase K**.

5. Mix and incubate for **15 min** at **50 °C**. Mix the samples by inverting the tubes a few times.

   Note: During viral proteolysis, viral DNA molecules pass into the solution. Carefully follow the sample to protect DNA from mechanical damage. The damaged molecules will not be functional in the transfection process.

6. During incubation prepare the Virus Mini AX columns placed inside 15 ml tubes.
Apply **800 µl** of **K1** equilibrating solution. Wait for the solution to flow through the column.

7. Apply the lysed virion solution onto the equilibrated column.
Wait for the lysate to flow through the column.

   The Virus Mini AX column works by means of gravity. The flow rate strongly depends directly on the quantity and size of DNA molecules in a sample. High content of high molecular weight DNA decreases the flow rate. DNA amount exceeding 15 µg loaded onto a column may lead to flow stoppage. In such cases the column should be centrifuged in a swing-out rotor for 1 min at 3000-4000 RPM. The centrifugation can be performed after the loading step (point 7) and during the washing step with K2 solution (point 8 and 9) or elution step with K3 solution (point 10).

8. Add **1.5 ml** of **K2** wash solution. Wait for the solution to flow through the column.

9. Add again **1.5 ml** of **K2** wash solution. Wait for the solution to flow through the column.

10. Add **100 µl** of **K3** elution solution. Wait for the eluate to flow through the column.

    Note. The purpose of this step is to decrease the total volume of eluate, since the column void volume is about 100 µl.

11. Transfer the column to a new **2 ml** tube (included).

    The column drop director possesses proper fitting that allows easy attachment to the precipitation tube.

12. Add **1 ml** of **K3** elution solution. Wait for the eluate to flow through the column.
Remove the column.

13. PM precipitation mix contains a precipitation enhancer and it should be intensively mixed before use by vigorous hand shaking.

Add **800 µl** of **PM** precipitation mix to the eluted DNA.

14. Mix the sample by inverting the tube a few times and centrifuge for **10 min** at **10 000 RPM**.

The light-blue DNA pellet should be visible at the bottom of the precipitation tube.

15. Carefully discard supernatant. Be careful not to remove the DNA pellet at the bottom of the tube.

Attention. When pouring out the supernatant, it is very easy to lose the DNA pellet. For safety, it is recommended to pour the supernatant into the prepared tube so the pellet can be recovered.

16. Add **500 µl** of **70% ethanol** (not included).
Mix the sample and centrifuge for **3 min** at **12 000 RPM.**

Note. The light-blue DNA pellet should be visible at the bottom of the precipitation tube.

17. Carefully discard supernatant. Be careful not to remove the DNA pellet at the bottom of the tube.

Attention. When pouring out the supernatant, it is very easy to lose the DNA pellet. For safety, it is recommended to pour the supernatant into the prepared tube so the pellet can be recovered.

18. Air dry the plasmid DNA pellet for **5 min** at **room temp**. up-site down.

Note. If there are any leftovers (small droplets) of alcohol on the tube walls they should be removed with sterile cotton buds.

Note: Do not prolong the DNA drying process. It may cause significantly insufficient DNA isolation capacity and thus subsequent transfection.

19. Dried DNA pellets can be dissolved in the desired volume of **TE** buffer or sterile water (not included).

Note. The blue color of DNA precipitate enables visual confirmation of the DNA dissolution process.

20. Store the DNA at -20 °C until later use.

Safety Information

DANGER

Proteinase K

H315 Causes skin irritation.
H319 Causes serious eye irritation.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335 May cause respiratory irritation.
P261 Avoid breathing dust.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P342+P311 If experiencing respiratory symptoms call a Poison Center or doctor/physician.

WARNING

L1.4E lysis solution

H302 Harmful if swallowed.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

WARNING

K1 equilibrating solution

H302 Harmful if swallowed.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

DANGER

K2 wash solution

H225 Highly flammable liquid and vapor.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

DANGER

K3 elution solution

H225 Highly flammable liquid and vapor.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

DANGER

PM precipitation mix

H225 Highly flammable liquid and vapor.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

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