A&A BIOTECHNOLOGY
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Manual

Genomic Mini AX Phage

Increased efficiency kit for genomic DNA purification from bacteriophages.
Procedure with DNA precipitation.

catalog #	size
011-20	20 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	011-20	storage
Columns	20 pcs	2–8 °C
2 ml tubes	20 pcs	15–25 °C
20 ml tubes	20 pcs	15–25 °C
PF precipitation solution	100 ml	15–25 °C
SM suspension solution	14 ml	15–25 °C
LSU lysis buffer	27 ml	15–25 °C
K1 equilibrating solution	22 ml	15–25 °C
K2 wash solution	88 ml	15–25 °C
K3 elution solution	28 ml	15–25 °C
PM precipitation mix	18 ml	15–25 °C
TE buffer	5 ml	15–25 °C
NM nucleases mix	330 µl	-20 °C
Proteinase K	600 µl	2–8 °C

The binding capacity of the minicolumn is 20 µg.

Additional equipment and reagents

Necessary

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

Protocol

1. Add **1/5 volume** of **PF** precipitation solution to phage lysates samples - proportion 5:1.
e.g. add 200 µl of PF solution to 1 ml of sample.

   Mix and keep on ice for **1 hour**.

2. Mix and centrifuge for **20 min** at **10 000 x g**.

   Mark the outer tube walls where the precipitated phage particles are supposed to be collected by centrifugation.

3. Discard the supernatant.
Suspend the phage pellet (invisible) in **600 µl** of **SM** suspension solution by washing thoroughly the wall of the tube.

4. Add **15 µl** of **NM** nucleases mix.

   Mix and incubate for **10 min** at **37 °C**.

5. Add **20 µl** of **proteinase K** and **1.2 m**l of **LSU** lysis buffer.

   Mix and incubate for **15 min** at **50 °C**.

6. During incubation prepare the columns for phage DNA purification. Place them inside 20 ml tubes and set in the suitable rack.

   Apply **1 ml** of **K1** equilibrating solution. Wait for the solution to flow through the column.

7. Apply the sample onto the equilibrated column.
Wait for the lysate to flow through the column.

8. Add **2 ml** of **K2** wash solution. Wait for the solution to flow through the column.

9. Add again **2 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 **5 min** at **10 000 x g**.

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 x g.**

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.

19. Dried DNA pellets can be dissolved in the desired volume of **TE** buffer.

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

LSU lysis buffer

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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