Extraction of DNA from Virus

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Abstract

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Guidelines

Materials:

1. Virus prep.
2. Lysing Solution (LS).
3. 5M Sodium Perchlorate (SP).
4. Mercaptoethanol.
5. Ethanol 76% & 95%.
6. Phenol – [Chloroform: isoamyl alcohol (25:1)] in the proportion equal to 1:1 .
7. TE : (10mM Tris-Hcl,1mM EDTA, pH 8.0 in 100ml water).
8. 3M or 5M Sodium Acetate.

Solutions:

Sodium Perchlorate , 5. M

In 70ml water : Dissolve anhydrous perchlorate (NaClO4; 61.2g/100ml)

Or monohydrate (NaClO4 H2O; 70.0g/100ml)

Bring to 100ml with water.

Make fresh for each use.

Lysing Solution (2x) (100mM Tris-HCl, 0.3 M NaCl, 20mM EDTA,

And 2 % (w/v) SDS,pH 8.0)

In 50ml of water :

Add 10ml of 1.0 M Tris-HCl (pH 8).

Add 20ml of 0.1M EDTA (pH 8).

Dissolve 1.8 g NaCl.

Dissolve 2.0g SDS (Sodium Dodecyl Sulfate).

Bring to 100ml with water. Store at 4ºC.

Ethanol, 76% (80% [v/v] aqueous solution made up using 95% ethanol)

Sodium Acetate, 3.0 M (pH 6.0)

In 80ml water ;

Dissolve 40.8g Trihydrate (NaC2H3O2 3H2O) or 24.6g Anhydrous (NaC2H3O2)

Adjust pH to 6.0

Bring to 100ml with water.

Isopropyl alcohol, 99%

Phenol-Chloroform (1:1 v/v)

1 volume Tris-HCl – saturated phenol (Fisher BP17501-400/BP1750B-26;

pH 6.6 + /-0.2 saturated with 1.0 M Tris-Hcl and 1mM EDTA)

! volume chloroform : isopentyl alcohol (25:1) Note : isopentyl alcohol = isoamyl alcohol.

Protocol

Step 1.
Mix Lysing Solution (LS) & Sodium Perchlorate (SP) in 2:1.

NOTES
Irina Agarkova 07 Apr 2016
Warning !!! This mixture forms a precipitate and must be warmed to 50-55ºC to redissolve. Add immediately before using 2 % of mercaptoethanol (2ml/150ml mixed LS+SP) & 10mg/100ml protease k to solution.

Step 2.
Mix the combined Lysing Solution & sodium Perchlorate with virus prep :18.5 ml LS+SP with 10ml

virus prep (10^{11} PFU/ml).

Step 3.
Incubate the lysate at 55ºC for 1hrs.

DURATION
01:00:00

Step 4.
Add 30ml of phenol : [chloroform : isoamyl alcohol (25:1)] in the proportion equal to 1:1.

Step 5.
Shake them by hands for 5mins.

DURATION
00:05:00

Step 6.
Transfer the liquid to the centrifuge tubes , balance them & centrifuge for 15 mins at 14000 rpm at 4ºC.

DURATION
00:15:00

Step 7.
Transfer the aqueous (upper) part with pipette to the clean centrifuge tubes. Don’t worry getting protein in it . Put the bottom layer into the waste container.

Step 8.
Centrifuge tubes again at 14,000 rpm for 15 min. Now it easy to get pure liquid without protein debris.

DURATION
00:15:00

Step 9.
Aqueous layer may be stored overnight at 4 ºC.

DURATION
18:00:00

Step 10.
Estimate the volume of the aqueous layer and add 0.1 volume of 3M (or 5M) Sodium Acetate (pH 6.0).

Step 11.
Gently overlay the aqueous layer with 2 volumes of 95 % of ethanol.

Step 12.
Collect DNA by swirling glass rod. Let it dry on the glass rod .

Step 13.
Pour off alcohol into the waste container.

Step 14.
Put DNA on the rod into the beaker & add 10 to 20ml of cold 76% Ethanol (-20ºC).

Step 15.
Let it stand for 10-20 min.

DURATION
00:20:00

Step 16.
Press the glass rod with DNA against the wall of beaker to get rid of the ethanol.

Step 17.
Invert the stirring rod in the test tube rack and dry for 5 min.

DURATION

00:05:00

Step 18.

Use 15ml plastic tubes. Dissolve DNA in 4ml of TE. Estimate DNA concentration using spectrophotometer or gel to ensure DNA quantity.