DNA/RNA Extractions from Sterivex Filters

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Abstract

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Guidelines

2.0 mL Lysis Buffer is added to Sterivex immediately after filtration. Filters are stored at -20°C or -80°C.

Lysis Buffer is:

Final Concentration	For 20 ml:
40 mM EDTA	1.6 ml of 0.5 M EDTA
50 mM Tris (pH 8.3)	1.0 of 1 M Tris (pH 8.3)
0.73 M Sucrose	5.13g sucrose

* Note: Must use Phenol:Chloroform:IAA (25:24:1) pH 8.0! Add buffer supplied, shake well, allow phases to separate and check pH of buffer using pH paper. Add hydroxyquinoline to a final concentration of 0.1% (100 mg hydroxyquinoline per 100ml phenol:chloroform:IAA).

Before start

Prepare lysis buffer as described in guidelines.

Protocol

Step 1.
Thaw sterivex filters on ice.

Step 2.
Add 40µL lysozyme solution (add 2 mg lysozyme to 40 µl Lysis Buffer) to filter.

AMOUNT
40 µl Additional info:

Step 3.
Incubate at 37°C while rotating for 45 minutes.

DURATION
00:45:00

Step 4.
Add 100µL Proteinase K solution (1mg Proteinase K in 100 µl Lysis Buffer) and 100µL 20% SDS.

AMOUNT
100 µl Additional info:

Step 5.
Incubate at 55°C while rotating for 2 hours.

DURATION
02:00:00

Step 6.
Transfer lysate to a 15 mL conical tube using a sterile 3 mL syringe.

Step 7.
Add 1 mL Lysis Buffer to filter and wash at 55°C for 15 minutes. Pool with above lysate.

AMOUNT
1 ml Additional info:

DURATION
00:15:00

PROTOCOL

. Lysis Buffer (20 mL)

CONTACT: Celina Gomez

Step 7.1.

1.6 mL of 0.5 M EDTA

REAGENTS
EDTA disodium dihydrate AB1011793 by Abblis

Step 7.2.

1.0 mL of 1 M Tris (pH 8.3)

REAGENTS
Tris RP-T60040 by P212121

Step 7.3.

5.13 g Sucrose

REAGENTS
Sucrose View by P212121

Step 8.
Add 3 mL Phenol:Chloroform:IAA (25:24:1; pH 8.0).

AMOUNT
3 ml Additional info:

NOTES
VERVE Team 18 Jun 2015

Must use Phenol:Chloroform:IAA (25:24:1) at pH 8.0. Add buffer supplied, shake well, allow the phases to separate and check pH of buffer using pH paper. Add hydroxyquinoline to a final concentration of 0.1% (100 mg hydroxyquinoline per 100 mL Phenol:Chloroform:IAA).

Step 9.
Vortex for 10 seconds.

DURATION
00:00:10

Step 10.
Spin for 5 minutes at 2500g (speed 8).

DURATION
00:05:00

Step 11.
Carefully transfer aqueous phase to a new 15 mL conical tube.

Step 12.
Add 3 mL Chloroform:IAA (24:1)

AMOUNT
3 ml Additional info:

REAGENTS
Chloroform:IAA C0549-1PT by Abblis

Step 13.
Vortex for 10 seconds.

DURATION
00:00:10

Step 14.
Spin for 5 minutes at 2500g (speed 8).

DURATION
00:05:00

Step 15.
Carefully transfer aqueous phase to a centricon 100.

Step 16.
All of the remaining volume should be transferred to an epi tube.

Step 17.
Spin Centricon at 1000xg (speed4) for 20 minutes.

DURATION
00:20:00

Step 18.
Add remaining volume from epi tube to centricon.

Step 19.
Spin until only 100µL - 500µL of aqueous phase is left in Centricon.

Step 20.
Add 1 mL TE Buffer

AMOUNT
1 ml Additional info:

Step 21.
Repeat step 20.

Step 22.
Spin until only 100 µL - 150 µL left in Centricon.

Step 23.
Carefully remove liquid without damaging the membrane.

Step 24.
Wash membrane well with 40µL of TE Buffer

AMOUNT

40 µl Additional info:

Step 25.
Pool the membrane in the same epi tube. Note the total volume in the epi tube.