QIAgen DNeasy Blood & Tissue kit, cultured cells

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Abstract

QIAgen's DNeasy Blood & Tissue kit (cat#69504 or 69506) for cultured cells

Citation: Harold Bien QIAgen DNeasy Blood & Tissue kit, cultured cells. **protocols.io**
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Before start

Preparation of buffer AW1 and AW2

Add ethanol to AW1 and AW2 as directed on bottle. Stable for one year.

If buffer AL has precipitates, warm to 56C until precipitates dissolved

Preheat thermoixer to 56C

Equipment required:

Pipettes and pipette tips
Vortexer
Microcentrifuge tubes, 1.5-1.7mL
Thermomixer for heating at 56C

Materials

- 1X PBS (Phosphate-buffered saline) by Contributed by users
- QIAgen DNeasy Blood and Tissue Kit, 50 rxn 69504 by Qiagen
- Ethanol, pure 4455 by Omnipure Filter Company
- Buffer AL, Lysis buffer 19076 by Qiagen
- Buffer AW1, Wash buffer (1), concentrate 19081 by Qiagen
- Buffer AW2, Wash buffer (2), concentrate 19072 by Qiagen
- Buffer AE, Elution buffer, 240mL 19077 by Qiagen

Protocol

Step 1.

Centrifuge a maximum of 5 million cells for 5 minutes at 300g

DURATION

00:05:00

Step 2.
Resuspend pellet in 200µL of PBS

AMOUNT
200 µl Additional info:

REAGENTS
1X PBS (Phosphate-buffered saline) by Contributed by users

Step 3.
Add 20µL proteinase K.

AMOUNT
20 µl Additional info:

REAGENTS
Proteinase K, 2mL 19131 by Qiagen

Step 4.
Add 200µL Buffer AL and mix thoroughly by vortexing

AMOUNT
200 µl Additional info:

REAGENTS
Buffer AL, Lysis buffer 19076 by Qiagen

Step 5.
Add 200µL pure ethanol and mix by vortexing

AMOUNT
200 µl Additional info:

REAGENTS
Ethanol, pure 4455 by Omnipure Filter Company

Step 6.
Pipet mixture into DNeasy Mini spin column placed on a 2mL collection tube (supplied with kit)

Step 7.
Centrifuge column at ≥6,000g for 1 minute

DURATION
00:01:00

Step 8.
Discard collection tube and flow through

Step 9.
Place spin column in new 2mL collection tube

Step 10.
Add 500µL buffer AW1

AMOUNT
500 µl Additional info:

REAGENTS
Buffer AW1, Wash buffer (1), concentrate 19081 by Qiagen

Step 11.
Centrifuge for 1 minute at≥6,000g

DURATION
00:01:00

Step 12.
Discard collection tube and flow through

Step 13.
Add 500µL of Buffer AW2

AMOUNT
500 µl Additional info:

REAGENTS
Buffer AW2, Wash buffer (2), concentrate 19072 by Qiagen

Step 14.
Centrifuge at 20,000g for 3 minutes

DURATION
00:30:00

Step 15.
Discard collection tube and flow through

Step 16.
Transfer spin column to new (final and labeled) collection tube [optionally sterilized]

Step 17.
Elute DNA by adding 200µL of buffer AE to the center of the spin column

AMOUNT
200 µl Additional info:

REAGENTS
Buffer AE, Elution buffer, 240mL 19077 by Qiagen

Step 18.
Incubate for 1 minute at room temperature

DURATION
00:01:00

Step 19.
Centrifuge at ≥6,000g for 1 minute

DURATION
00:01:00

Step 20.
[Optiona]: Repeat step 19 for increased DNA yield