Transiom Sputum DNA/RNA kit

Cat No: TSPK-50/100

Contents

Introduction

Transiom Sputum DNA/RNA Isolation Kit provides a rapid procedure for the isolation of sputum DNA/RNA. A sputum specimen is the name given to the mucus which is expectorated from the lower airways. The best sputum samples will contain very little saliva, as this contaminates the sample with oral bacteria. Sputum samples are typically evaluated to look for infections such as *Moraxella catarrhalis*, *Mycobacterium tuberculosis, Streptococcus pneumoniae Respiratory syncytial virus (RSV), rhinovirus (RV), human bocavirus (HBoV), human metapneumovirus (HMPV) Influenza, parainfluenza viruses* and *Haemophilus influenza*. Other pathogens can also be detected in sputum. In addition, sputum DNA can be evaluated for the detection of lung cancer or to evaluate chronic inflammation.

Transiom Sputum DNA/RNA Isolation Kit constituents an all-in-one system for the isolation of DNA from sputum samples. The kit allows for the isolation of bacterial or eukaryotic DNA from the sputum samples using spin-column chromatography based on Transiom proprietary resin. The genomic DNA is isolated free from inhibitors, and can then be used as the template in a number of downstream assays

The Transiom™ Sputum DNA/RNA Kit uses a simple four-step method:

1. Effectively disrupting or homogenizing the starting material to release the DNA/RNA.
2. Binding DNA/RNA to the TransPure™ Binding Column.
3. Removing impurities with wash solution.
4. Eluting purified DNA/RNA.

Storage and Stability

All components of the Sputum DNA/RNA kit are stable for at least 12 months at storage condition from date of purchase. Proteinase K, Poly A and Lysozyme should be stored at -20°C. During shipment, or storage in cool ambient conditions, precipitates may form in Buffer SPL and Buffer TW. It is possible to dissolve such deposits by warming the solution at 50°C, though we found that they do not inter-fare with overall performance.

Product components and Storage conditions:

Product	TSPK-50	TSPK-100	Storage
Preps	50	100	-
Buffer SPL	30.0 ml	60.0 ml	RT
Buffer TW	15.0 ml	30.0 ml	RT
Buffer TW I	10.0 ml	20.0 ml	RT
Elution Buffer	10.0 ml	20.0 ml	RT
Poly A	2 mg	4 mg	-20^{0}C
Proteinase K	15 mg	30 mg	-20^{0}C
Lysozyme	20 mg	40 mg	-20^{0}C
TransPure™ Column	50 nos.	100 nos.	RT
Collection Tubes	50 nos.	100 nos.	RT
User manual	1	1	-

Before Starting

- Read carefully all manual instructions before starting
- Proteinase K should be store at -20°C
- Lysozyme should be store at -20°C
- Poly A should be store at -20°C

Important:

For 50 extraction kit:

☑ Dilute **Buffer TW** with **absolute ethanol** as follows and store at room temperature:

Buffer TW: Add 15 ml absolute (96%-100%) ethanol per bottle

☑ Dilute **Buffer TW I** with **absolute ethanol** as follows and store at room temperature:

Buffer TW I: Add 40 ml absolute (96%-100%) ethanol per bottle

☑ Add **1 ml Elution Buffer** in **Proteinase K** containing vial.

☑ Add **300µl Elution Buffer** in **Poly A** containing vial.

☑ Add **1 ml Elution Buffer** in **Lysozyme** containing vial

For 100 extraction kit:

☑ Dilute **Buffer TW** with **absolute ethanol** as follows and store at room temperature:

Buffer TW: Add 30 ml absolute (96%-100%) ethanol per bottle

☑ Dilute **Buffer TW I** with **absolute ethanol** as follows and store at room temperature:

Buffer TW I: Add 80 ml absolute (96%-100%) ethanol per bottle

☑ Add **2 ml Elution Buffer** in **Proteinase K** containing vial.

☑ Add **600 µl Elution Buffer** in **Poly A** containing vial.

☑ Add **2 ml Elution Buffer** in **Lysozyme** containing vial

Sputum DNA/RNA Kit Protocol

1. **Take 350 µl of Sputum sample and transfer it to sterile 1.5 ml eppendorf tube.**

Note: The quality and quantity of DNA/RNA depend upon the age and storage of sputum samples.

2. **Add 200 µl of TE Buffer (Tris EDTA Buffer pH-8.0/ TE Buffer) to the eppendorf tube. Add 20 µl of Lysozyme Enzyme to the eppendorf tube, mix it well by vortexing and incubate at**

37°C/ Room Temperature for 20 mins.

3. Add 500µl of Buffer SPL and also add 20µl Proteinase K and 5 µl Poly A Mix thoroughly by vortexing.
4. Incubate at 60°C for 15-20 minutes either on thermo mixer or water bath or dry bath. Briefly invert the tube 4-5 times during incubation.
5. After incubation add 300µl of 96-100% ethanol to the lysate. Mixed by brief vortex for 10 seconds.
6. Transfer 650µl lysate to the spin column, and centrifuge at 10,000 rpm for 1 mins. Discard the flow through liquid.
7. Repeat above step, until the entire sample has been processed and retain column for further processing.
8. Place the column into the same collection tube. Add 500µl of Buffer TW. Centrifuge at 10,000 rpm for 1 min. discard the flow through.
9. Place the column into same collection tube. Add 500µl of TW I. Centrifuge at 10,000 rpm for 1 min. discard the flow through.
10. Repeat the Step-9.
11. Place empty DNA spin column, into the same collection tube and centrifuge at 10,000 rpm for 2 min.
12. Place the column into a new sterile 1.5 ml eppendorf tube, add 30 µl preheated (In case of DNA) Elution buffer. Incubate at room temperature for 5 min. (perform this step twice

30 µl + 30 µl = 60 µl)

Note: 1. For RNA Extraction Pre Heated Elution Buffer not required.

2. For RNA Extraction Incubate at Room Temperature Only.

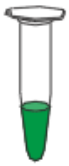
3. For RNA Extraction reduce the elution volume 20 µl + 20 µl = 40 µl or lower elution volume as per Downstream process.

13. Centrifuge 10,000 rpm for 1 min to elute pure Sputum DNA/RNA. The first elution normally yields 60-70 % of DNA/RNA bound. A second elution with another 30 µl/ 20 µl buffer will yield another 20 % of the DNA/RNA.

Note: Elution volume may vary as per downstream process

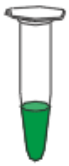
14. Discard the Column, and save elute. Do not reuse binding columns or collection tubes.

FLOW CHART



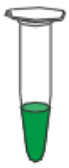
Preparation of samples

- Take 350µl Sputum sample



Lysis

- Add 200µl of TE Buffer and mixed with 20µl of Lysozyme.
- Incubate at 37 ^{0}C/ RT for 20 mins.
- Add 500µl of Buffer SPL and mixed with 20µl of Proteinase K and 5 µl Poly A



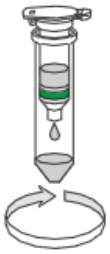
Incubation & Phase Separation

- Incubate at 60 ^{0}C for 15-20 mins.

Binding to silica column

- Adjust binding of phase with ethanol

Hands on time 30 mins



Washing

- Washing twice with wash buffer TW and TW I
- Dry column

Elution of Pure DNA/RNA

Troubleshooting

Problems	Possible reason	Suggestions
Clogged column	Incomplete Lysis	Add the correct volume of Buffer SPL and incubate for specified time at 60°C. It may be necessary to extend incubation time by 10 min.
	Sample is too high	If using more than 400 µl of Sputum Sample increase volume of Proteinase K, Buffer SPL and Ethanol. Pass aliquots of lysate through one column successively.
	Sample is too viscous	Divide sample into multiple tubes and adjust the volume to 400 µl with 10mM Tris -HCL.
Low DNA/RNA Yield	Clogged Column	See above
	Poor elution	Repeat elution or increase elution volume. Incubation of column at 70°C for 5 min with elution buffer may increase yields.
	Improper washing	Wash buffer concentrate must be diluted with ethanol.
Low A260/280 Ratio	Extended centrifugation during elution	Resin from the column may be present in elute and effect the OD absorbance. Avoid centrifugation at speed higher than 12,000 rpm.
	Poor cell lysis due to incomplete mixing with buffer SPL	Repeat the procedure, this time making sure to vortex the sample with Buffer SPL immediately and completely.
No DNA/RNA Eluted	Poor Cell Lysis due to improper mixing with Buffer SPL	Mix thoroughly with Buffer SPL.
	No Ethanol added to wash buffer concentrate.	Dilute Wash buffer with the indicated volume of absolute ethanol before use.
Eluted Material has Red or Brown Color	Sample volume is too high	Reduce sample volume and Proceed with protocol.

Limited Use and Warranty

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