Transiom™ Universal Clinical Samples Nucleic Acid Extraction Kit

Cat No: TCNE-50/100/200

Contents

Introduction

The Transiom™ Universal Clinical Samples Nucleic Acid Extraction Kit provides a fast, simple technique for preparation of purified and intact Nucleic Acids from Blood, Plasma, Serum Bronchoalveolar lavage fluid (BAL), Sputum, Tissue/Biopsy, Urine, peritoneal fluid, Pleural fluid, pericardial fluid, Cerebrospinal fluid (CSF), Amniotic Fluid, Pus, Synovial fluid, Fungal Liquid Culture, Slants and Culture Plate, etc. Transiom™ Universal Clinical Samples Nucleic Acid Extraction kit can generally used to detect Fungal, Bacterial and Viral Infections from above mentioned Clinical Samples. The kit combines the advantages of silica binding with a TransPure™ Spin Column.

The system combines the reversible nucleic acid-binding properties of the matrix with the speed and versatility of spin column technology to yield high quality of Nucleic Acids with the OD260/OD280 ratio of 1.8-2.2. Purified Nucleic Acids is suitable for PCR, restriction digestion, NGS, hybridization techniques and others.

The Transiom™ Universal Clinical Samples Nucleic Acid Extraction Kit uses a simple four-step method:

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

Storage and Stability

All components of the Transiom™ Universal Clinical Samples Nucleic Acid Extraction Kit are stable for at least 12 months at storage condition from date of purchase. Proteinase K and Carrier RNA should be stored at -20°C. During shipment, or storage in cool ambient conditions, precipitates may form in Buffer TCSL and Buffer TW. It is possible to dissolve such deposits by warming the solution at 50°C-55°C, though we found that they do not inter-fare with overall performance.

Product components and Storage conditions:

Product	TCNE-50	TCNE-100	TCNE-200	Storage
Preps	50	100	200	-
Buffer TTL	10.0 ml	20.0 ml	30.0 ml	RT
Buffer TCSL	30.0 ml	60.0 ml	120.0 ml	RT
Buffer TW	15.0 ml	30.0 ml	60.0 ml	RT
Buffer TW I	14.0 ml	28.0 ml	55.0 ml	RT
Elution Buffer	10.0 ml	20.0 ml	40.0 ml	RT
Carrier RNA (lyophilized)	5.0 mg	10.0 mg	15.0 mg	-20°C
Proteinase K (lyophilized)	33 mg	33 mg × 02	132 mg	-20°C
Lysozyme (lyophilized)	10 mg	20 mg	40 mg	-20°C
TransPure™ Column	50 nos.	100 nos.	200 nos.	RT
Collection Tubes	50 nos.	100 nos.	200 nos.	RT
User manual	1	1	1	-

Before Starting

- Read carefully all manual instructions before starting
- Proteinase K should be store at -20°C
- Carrier RNA should be store at -20°C

Important:

For 50 Extractions

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

Buffer TW: Add **15 ml** absolute (99%-100%) ethanol per bottle

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

Buffer TW I: Add **56 ml** absolute (99%-100%) ethanol per bottle

☑ Add **1100µl Elution Buffer** in **Proteinase K** containing vial. (Store at -20°C after reconstitute)

☑ Add **300µl Elution Buffer** in **Carrier RNA** containing vial. (Store at -20°C after reconstitute

☑ Add **250µl Elution Buffer** in **Lysozyme** containing vial. (Store at -20°C after reconstitute)

For 100 Extractions

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

Buffer TW: Add **30 ml** absolute (99%-100%) ethanol per bottle

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

Buffer TW I: Add **112 ml** absolute (99%-100%) ethanol per bottle

☑ Add **1100µl Elution Buffer** in **Proteinase K** containing vial **(× 2 Vials)** (Store at -20°C after reconstitute)

☑ Add **600µl Elution Buffer** in **Carrier RNA** containing vial. (Store at -20°C after reconstitute)

☑ Add **500µl Elution Buffer** in **Lysozyme** containing vial. (Store at -20°C after reconstitute)

For 200 Extractions

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

Buffer TW: Add **60 ml** absolute (99%-100%) ethanol per bottle

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

Buffer TW I: Add **220 ml** absolute (99%-100%) ethanol per bottle

☑ Add **4400µl Elution Buffer** in **Proteinase K** containing vial. (Store at -20°C after reconstitute)

☑ Add **1200µl Elution Buffer** in **Carrier RNA** containing vial. (Store at -20°C after reconstitute)

☑ Add **1000µl Elution Buffer** in **Lysozyme** containing vial. (Store at -20°C after reconstitute)

❖ EDTA Blood, EDTA Plasma / Serum Nucleic Acid Extraction Protocol:

1. Thoroughly mix the blood sample for at least 10 minutes in a rotisserie shaker at room temperature. If the blood has been frozen, thaw completely before mixing for 10 minutes.

2. Pipet 20 µl of Proteinase K and 5 µl of Carrier RNA to sterile 1.5 ml eppendorf tube.

3. Add 550 µl of Buffer TCSL in eppendorf tube.

4. Take 250 µl of fresh EDTA blood/ Plasma/ Serum sample and transfer it to eppendorf tube and Mix thoroughly by vortexing for 30 seconds.

Note: The quality and quantity of Nucleic Acids depend upon the age and storage of blood samples. Mix well blood sample through pipetting and then proceed.

5. Incubate at 70°C for 20 minutes either on thermo mixer or water bath or dry bath. Briefly invert the tube 5-7 times during incubation.

Note: To ensure efficient lysis, it is essential that the sample and Buffer TCSL are mixed thoroughly to yield a homogeneous solution.

6. After incubation add 250 µl volume of 99-100% ethanol to the lysate. Mixed by inverting a tube 15- 20 times or Pulse Vortexing. Incubate for 30 seconds at room temperature (18–25°C).

7. Transfer 650µl lysate to the DNA spin column, and centrifuge at 12,000 rpm for 2 mins. Discard the flow through liquid.

8. Repeat above step, until the entire sample has been processed and retain column for further processing.

9. Place the column into the same collection tube. Add 500µl of Buffer TW. Centrifuge at 12,000 rpm for 1 min. discard the flow through.

10. Place the column into same collection tube. Add 650µl of TW I. Centrifuge at 12,000 rpm for 1 min. discard the flow through.

11. Place empty DNA spin column, into the same collection tube and centrifuge at 12,000 rpm for 2 min.

12. Place the column into a new sterile 1.5 ml eppendorf tube, add 40 µl Elution buffer. Incubate at room temperature for 2 min. Centrifuge 12,000 rpm for 1 min to elute pure Nucleic Acids.

13. Discard the Column, Keep eluted Nucleic Acids on ice or store at -20°C.

❖ Bronchoalveolar lavage fluid (BAL), Cerebrospinal fluid (CSF), Amniotic Fluid, Peritoneal fluid, Pleural fluid, Pericardial fluid, Urine, Fungal/Bacterial Liquid Culture, Slants and Culture Plate Nucleic Acid Extraction Protocol:

1. Transfer Sample into a 2.0 ml microfuge tube (provided by user) as per following

Bronchoalveolar lavage fluid (BAL) - 500 µl- 1.0 ml
Cerebrospinal fluid (CSF) - 500 µl- 1.0 ml
Amniotic Fluid - 500 µl- 1.0 ml
Peritoneal fluid - 500 µl- 1.0 ml
Pleural fluid - 500 µl- 1.0 ml
Pericardial fluid- 500 µl- 1.0 ml
Urine- 2.0 ml
Fungal/ Bacterial Liquid Culture- 2.0 ml
Note: For Fungal /Bacterial Liquid culture centrifuge the culture at 14,000 rpm for 5 min and wash the pellet with 1X TE buffer or 1X PBS buffer 2 times with resuspend the pellet in 1X TE Buffer or 1X PBS Buffer. After this step proceed further with step-2 (Lysis Step)

Note: 1. Use sample as per availability. Can use sample from 500 µL - 2.0 ml range.
2. Higher sample input can enhance the detection of assay.

For Slants and Culture Plate: (Start Procedure from this step)
Take loopful of culture using micro tip or sterilized inoculation loop. Resuspend it in following mixture of Proteinase K, Carrier RNA and Buffer TCSL. (Step-4)

2. Add 20 µl of Proteinase K, 5 µl of Carrier RNA and 550 µl of Buffer TCSL to the microfuge tube, Resuspend the cell pellet by pipetting.
Note: To ensure efficient lysis, it is essential that the sample and Buffer TCSL are mixed thoroughly to yield a homogeneous solution.

3. Incubate at 70°C for 20 minutes either on thermo mixer or water bath or dry bath. Briefly invert the tube 5-7 times during incubation.

4. After incubation add 350 µl volume of 99-100% ethanol to the lysate. Mixed by inverting a tube 15- 20 times or Pulse Vortexing. Incubate for 30 seconds at room temperature.

5. Transfer 650µl lysate to the DNA spin column, and centrifuge at 12,000 rpm for 2 mins. Discard the flow through liquid.

6. Repeat above step, until the entire sample has been processed and retain column for further processing.

7. Place the column into the same collection tube. Add 500µl of Buffer TW. Centrifuge at 12,000 rpm for 1 min. discard the flow through.

8. Place the column into same collection tube. Add 650µl of TW I. Centrifuge at 12,000 rpm for 1 min. discard the flow through.

9. Again place the column into same collection tube. Add 650µl of TW I. Centrifuge at 12,000 rpm for 1 min. discard the flow through.

10. Place empty DNA spin column, into the same collection tube and centrifuge at 12,000 rpm for 2 min.

11. Place the column into a new sterile 1.5 ml eppendorf tube, add 40 µl Elution buffer. Incubate at room temperature for 2 min. Centrifuge 12,000 rpm for 1 min to elute pure Nucleic Acids.

12. Discard the Column, Keep eluted Nucleic Acids on ice or store at -20°C.

❖ Tissue/Biopsy Nucleic Acid Extraction Protocol:

1. Cut up to 50-100 mg tissue/ biopsy into small pieces, and place in a 2.0 ml micro centrifuge tube. Add 350 µl Buffer TTL.

Note: We strongly recommend cutting the tissue into small pieces to enable more efficient lysis. If desired, lysis time can be reduced by grinding the sample in liquid nitrogen before addition of Buffer TTL and Proteinase K.

2. Add 40 µl Proteinase K and Mix thoroughly by vortexing, and incubate at 70°C until the tissue is completely lysed. Briefly invert the tube during incubation.

Note: Approximate 45-70 minutes depend on sample source. Check tissue lysis during the incubation. Pulse Vortexing the tube 2-3 times during incubation for efficient lysis. If tissue is

completely lysed go for further step, higher incubation time after lysis of tissue may cause degradation of the Nucleic Acids. The quality and quantity of Nucleic Acids depend upon the age and storage of Tissue samples.

3. **Add 300 µl Buffer TCSL and 5 µl Carrier RNA to the sample, Vortex for 15-20 seconds and incubate at 65°C for 10 min and mix thoroughly by inverting. Then add 200 µl ethanol (99–100%), Mixed by inverting a tube 15- 20 times or Pulse Vortexing. Incubate for 1 min at room temperature (18–25 °C). Briefly centrifuge the Microfuge tube (~ 1-2 s at ~ 2,000 x g) to remove drops from the lid (short spin only).**

4. **Transfer 650µl lysate to the DNA spin column, and centrifuge at 12,000 rpm for 2 min. Discard the flow through liquid.**

5. **Repeat above step, until the entire sample has been processed and retain column for further processing.**

6. **Place the column into the same collection tube. Add 500µl of Buffer TW. Centrifuge at 12,000 rpm for 1 min. discard the flow through.**

7. **Place the column into same collection tube. Add 650µl of TW I. Centrifuge at 12,000 rpm for 1 min. discard the flow through.**

8. **Again place the column into same collection tube. Add 650µl of TW I. Centrifuge at 12,000 rpm for 1 min. discard the flow through.**

9. **Place empty DNA spin column, with the lid opens into the same collection tube and centrifuge at 12,000 rpm for 2 min.**

10. **Place the column into a new sterile 1.5 ml eppendorf tube, add 40 µl Elution buffer. Incubate at room temperature for 2 min. Centrifuge 12,000 rpm for 1 min to elute pure Nucleic Acids.**

11. **Discard the Column, Keep eluted Nucleic Acids on ice or store at -20°C.**

❖ Pus sample, Synovial fluid and Sputum Nucleic Acid Extraction Protocol:

1. **Transfer Sample into a 2.0 ml microfuge tube (provided by user) as per following**
   Pus Sample: 150 µl
   Synovial Fluid: 200 µl
   Sputum Sample: 200 µl
   Note: The quality and quantity of DNA/RNA depend upon the age and storage of sputum samples.

2. **Add 300 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 Room Temperature for 15 mins.**

3. **Add 550µl of Buffer TCSL and also add 20µl Proteinase K and 5µl Carrier RNA Mix thoroughly by vortexing.**

4. **Incubate at 70°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 12,000 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 12,000 rpm for 1 min. discard the flow through.**

9. **Place the column into same collection tube. Add 650µl of TW I. Centrifuge at 12,000 rpm for 1 min. discard the flow through.**

10. **Again place the column into same collection tube. Add 650µl of TW I. Centrifuge at 12,000 rpm for 1 min. discard the flow through.**

11. **Place empty DNA spin column, into the same collection tube and centrifuge at 12,000 rpm for 2 min.**

12. **Place the column into a new sterile 1.5 ml eppendorf tube, add 40 µl Elution buffer. Incubate at room temperature for 2 min. Centrifuge 12,000 rpm for 1 min to elute pure Nucleic Acids.**

13. Discard the Column, Keep eluted Nucleic Acids on ice or store at -20°C.

Trouble shooting guide:

Problems	Possible reason	Suggestions
Clogged column	Incomplete Lysis	Add the correct volume of Buffer TCSL or Buffer TTL and incubate for specified time at 68°C. It may be necessary to extend incubation time by 10 min- 40 min.
	Sample is too high	If using more than mentioned specified sample volume, increase volume of Proteinase K, Buffer TCSL Buffer TTL 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 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 TCSL Buffer TTL	Repeat the procedure, this time making sure to vortex the sample with Buffer TCSL immediately and completely.
No DNA Eluted	Poor Cell Lysis due to improper mixing with Buffer TCSL	Mix thoroughly with buffer TCSL.

	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

This product is intended for in vitro research use only. Transiom guarantees the performance of all products in the manner described in our product literature. We reserve the right to change, alter, or modify any product to enhance its performance and design. No other warranties of any kind express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Transiom, Transiom sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Transiom, to replace the products, Transiom shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product. This product is warranted to perform as described in its labeling and in Transiom's literature when used in accordance with instructions. If a Transiom product does not meet your expectations, simply call Transiom Technical Support Team.

For technical support or for more product information, please visit our website at www.transiom.co.in