T-Pro Genomic DNA Midi Kit (20)
For blood

Store at RT

(RB94-NGM020) 20 preps

T-Pro Biotechnology

This product is for laboratory research ONLY and not for diagnostic use.

Description	T-Pro Genomic DNA Midi Kit provides a fast and economical method for purification of total DNA (including genomic, mitochondrial and viral DNA) from whole blood(fresh), plasma, serum, buffy coat, other body fluids, lymphocytes, and cultured cells. There is no requirement for phenol/chloroform extraction or alcohol precipitation. Purified DNA is suitable for PCR or other enzymatic reactions.
Properties	- Complete removal of all contaminants for reliable downstream applications. - No phenol, chloroform or alcohol. - Rapid and simple procedure.
Applications	Purified DNA are ready for direct use in PCR, Southern Blotting, Real-Time PCR, AFLP, RFLP, PADP.
Quality Control	The quality of T-Pro Genomic DNA Midi Kit is tested on a lot – to-lot basis. The kit is tested by isolation of genomic DNA from 2 ml of human whole blood. Purified DNA is quantified with a spectrophotometer and the yield of genomic DNA is 40-60 µg with A260/A280 ratio 1.7 to 1.9.
Storage	T-Pro Genomic DNA Midi Kit is stable for RT

T-Pro Genomic DNA Midi Kit	
	RB94-NGM020
NB Buffer	75 ml
W1 Buffer	45 ml
W2 Buffer *	25 ml
Elution Buffer	30 ml
Proteinase K **	4 x 11 mg
GS Columns	100 pcs

Sample size: Up to 3 ml
Fresh/Frozen Blood
Yield: Up to 200 µg
Format: Spin Columns
Operation: Centrifuge or Vacuum
Operation Time: 20-30 Minutes

*Add 100 ml ethanol (96-100%) to W2 Buffer prior to the initial use.
**Add 1.1 ml ddH2O to a Proteinase K tube, vortex to dissolve. Store prepared Proteinase K at 4℃.

T-Pro Genomic DNA Midi Kit

Blood Protocol

1. **Add 100 ml ethanol (96-100%) to W2 Buffer prior to the initial use.**
2. **When the sample is low-copy(<10,000 copies), addition 10 µl of an aqueous solution Carrier DNA(10 µg/µl) to 3 ml NB Buffer before use.**
3. **Additional requirements:**
   - **50 ml centrifuge tube**
   - **Proteinase K (10 mg/ ml)**
   - **Ethanol (96-100%)**
   - **RNase A (50 mg/ ml)**
4. **Carrier DNA(e.g., poly dA, poly dT, poly dA:dT), concentration: 10 µg/µl.**

Cell Lysis	1	Apply up to 2 ml of blood or 10^7 of cultured cells to a 15 ml centrifuge tube(not provided). If sample volume is less than 2 ml, add the appropriate volume of PBS.
	2	Add 200 µl Proteinase K (10 mg/ ml) to the tube and mix by vortexing.
	3	Incubate the mixture at room temperature for 15 minutes. During incubation, invert the tube every 3 minutes.
	4	Add 3 ml NB Buffer to the tube and mix by vortexing.
	5	Incubate the mixture in a 60℃ water bath for 15 minutes. During incubation, invert the tube every 3 minutes.
	6	At this time, preheat required Elution Buffer (1 ml per sample) in a 60℃ water bath (For DNA Elution). Optional Step: RNA degradation If RNA-free genomic DNA is required, perform this optional step. Add 4 µl of RNase A (50 mg/ ml, provided by user) to sample lysate and mix by vortexing. Incubate at room temperature for 5 minutes.
DNA Binding	7	Add 3 ml of ethanol (96-100%) to the sample lysate and mix immediately by vortexing for 10 seconds.
	8	Place a GM Column on a 50 ml centrifuge tube (provided by user).
	9	Apply all the mixture (including any precipitate) from previous step to the GM Column.
	10	Close the cap and centrifuge at 4000 × g for 5 minutes.
Wash	11	Add 2 ml of W1 Buffer into the GM Column.
	12	Centrifuge at 4,000 x g for 3 minutes.
	13	Discard the flow-through and place the GM Column back in the 50 ml centrifuge tube.
	14	Add 4 ml of W2 Buffer (ethanol added) into the GM Column.
	15	Centrifuge at 4,000 x g for 3 minutes.
	16	Discard the flow-through and place the GM Column back in the 50 ml centrifuge tube.
	17	Centrifuge at 4,000 x g for 10 minutes to dry the column matrix.
DNA Elution	18	Standard elution volume is 500 µl. If less sample to be used, reduce the elution volume (250 µl) to increase DNA concentration.
	19	Transfer dried GM Column into a clean the 50 ml centrifuge tube (not provided).
	20	Add 500 µl of preheated Elution Buffer into the center of the column matrix.
	21	Stand for 5 minutes until Elution Buffer absorbed by the matrix.
	22	Centrifuge at 4,000 x g for 3 minutes to elute purified DNA.
	23	If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to about 1 ml.