TANBead® Nucleic Acid Extraction Kit

OptiPure cfDNA Auto Kit

(For use with the Maelstrom 2410 series)

CE IVD

L91C045

(For Professional Use Only) V4

1. Intended Purpose

The TANBead® Nucleic Acid Extraction Kit is a nucleic acid purification kit based on magnetic bead technology by using with corresponding TANBead® Nucleic Acid Extractor, which can automatically isolate and purify cell-free DNA (cfDNA) from human serum and plasma. The purified cfDNA can be used with any downstream application employing PCR-based qualitative, semi-quantitative, quantitative assays and capillary electrophoresis. The kit is intended for use by technicians, physicians, and biologists with well-trained in molecular biological techniques, the techniques of magnetic bead purification and *in vitro* diagnostic procedures. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted related to other clinical or laboratory findings. The kit is not limited to any specific disorder, condition, or other additional accompanying diagnostics. It is applicable for all population.

2. The basic principle

The silicon dioxide layer coated on the magnetic beads can adsorb the negatively charged molecules to purify nucleic acids from samples.

3. Specification

Starting Materials	1~6 mL plasma / serum
Elution Volume	50~100 µL
Typical DNA yield	1~100 ng per mL plasma / serum
Typical DNA size	100~300 bp

⚠ **DNA yield will vary from 1~100 ng according to sample source and storage method. The average DNA yield is 20 ng per mL of serum from healthy individuals.**

4. Component Supplied with the Kit

Lysis buffer	120 mL x 4	Guanidine salt, tris buffer, surfactant
Washing buffer 1	60 mL x 1	Guanidine salt, tris buffer, pH 8.0
Washing buffer 2	24 mL x 1	Add 96 mL 95~100% EtOH before use
Magnetic Beads	1.5 mL x 2	Magnetic Beads
Elution Buffer	1.5 mL x 4	Nuclease-Free Water
Proteinase K	1.0 mL x 12	Proteinase K, store at 2~8 °C
Protocol	1	Instruction guide for user

5. Auto Plate Content and Plate Position

Plate Position	Buffer	Volume (µL)
1	Lysis Buffer	As described in Chapter 11
2	Lysis Buffer	As described in Chapter 11
3	Washing Buffer 1	1000 µL
4	Washing Buffer 2	1000 µL
5	Washing Buffer 2	1000 µL
6	Elution Buffer	50~100 µL
7	N / A	N / A
8	Spin Tip	-

6. Kit Storage and Shelf Life

1) Components under room temperature (15~35°C) can be stored until the expiration date labeled on the box.
2) The proteinase K is transported at room temperature. Upon received, please store proteinase K at 2~8°C.

7. Precautions

1) It can be used for *in vitro* diagnostic.
2) When the temperature is below 20°C, place the reagent plate in an oven (preheated 42~60°C) from 5 to 10 minutes.
3) Avoid vigorous shaking, in order to avoid excessive formation of foam.
4) Carefully remove aluminum foil to avoid splashing.
5) Do not expose the opened reagents or Auto Plates / Auto Tubes to air. The evaporation would lead to pH change, or effect on the extraction effectiveness.
6) The reagents are all colorless and transparent. Colored reagents indicate contamination, please replace it with a fresh Auto Plates / Auto Tubes before proceeding.
7) Please check the integrity of the Auto Plates / Auto Tubes and remember to mount the spin tips into the appropriate position of the suitable instrument before operating them.
8) Please wear a mask and disposable gloves when handling.
9) Use sterile consumables to avoid nuclease contamination.
10) Reagent solution contains guanidine salt, avoid using bleach containing detergent.
11) Avoid eyes, skin, and clothing contact with reagents. In case of any contact, flush with flowing water.
12) If any serious incident occurs, please report to the manufacturer and the competent authority of the member state in which the user and / or the patient is established.

8. Materials required, Not Supplied

1) TANBead® Nucleic Acid Extraction System
   Model: Maelstrom 2410 series (non-sterile)
2) Disposable gloves
3) Scissors, utility knives
4) Micropipette, disposable tips (10 µL / 200 µL / 1000 µL)
5) 1.5 mL microcentrifuge tube
6) 15 mL / 50 mL conical tube
7) 20% SDS (Sodium dodecyl sulfate)
8) Streck Cell-Free DNA BCT tubes or other Cell-Free DNA collection tube

9. Sample Collection, Storage and Transportation

■ **Sample collection and storage**

1) Plasma
   a. Blood samples collected in Streck Cell-Free DNA BCT tubes is recommended. It is stable for up to 14 days.
   b. Centrifuge whole blood at **1600 x g** for **10 minutes**.
   c. Transfer the upper plasma layer to a new conical tube.
   d. Centrifuge at **16000 x g** for **10 minutes**.
   e. Carefully transfer the supernatant to a new conical tube for cfDNA isolation.
2) Serum
   a. Blood samples collected in serum separator tube.
   b. Let the blood clot for at least **30 minutes** in room temperature.
   c. Centrifuge whole blood at **1600 x g** for **10 minutes**.
   d. Transfer the upper serum layer to a new conical tube.
   e. Centrifuge at **16000 x g** for **10 minutes**.
   f. Carefully transfer the supernatant to a new conical tube for cfDNA isolation.
3) Specimen storage
   a. After centrifugation, the plasma / serum can be stored at
      i. 2~8°C up to 7 days.
      ii. -20°C for long-term preservation.

■ **Specimen transportation**

Transportation of whole blood, plasma and serum specimens should be followed by specific pathogen transportation-related regulations. The whole blood sample should be kept between 2~25°C during transportation and within 6 hours for separated serum. Plasma/ Serum sample can be transported between 2~8°C or by frozen.

10. Nucleic Acids Extraction Protocol

1) Mix sample with **20% SDS** and **proteinase K** as described in below table.

Sample volume	20% SDS	Proteinase K	Sample volume	20% SDS	Proteinase K
1 mL	60 µL	40 µL	4 mL	240 µL	160 µL
2 mL	120 µL	80 µL	5 mL	300 µL	200 µL
3 mL	180 µL	120 µL	6 mL	360 µL	240 µL

2) Mix well and incubate at **60°C** for **20 minutes**.
3) Load reagent buffers into 24-well plates as described in below table.

Plate No.	Buffer	Sample Volume			Plate No.	Buffer	Sample Volume		
		1 mL	2 mL	3 mL			4 mL	5 mL	6 mL
1	LB	1600 µL	3200 µL	4800 µL	1	LB	3200 µL	4000 µL	4800 µL
	MB	30 µL				MB	30 µL		
2					2	LB	3200 µL	4000 µL	4800 µL
						MB	30 µL		
3	WB1	1000 µL			3	WB1	1000 µL		
4	WB2	1000 µL			4	WB2	1000 µL		
5	WB2	1000 µL			5	WB2	1000 µL		
6	EB	50 - 100 µL			6	EB	50 - 100 µL		

4) Add sample into Auto Plate as described in below table.

Sample volume	Plate 1	Plate 2	Sample volume	Plate 1	Plate 2
1 mL	1 mL		4 mL	2 mL	2 mL
2 mL	2 mL		5 mL	2.5 mL	2.5 mL
3 mL	3 mL		6 mL	3 mL	3 mL

5) Select a program "**CFDNA-SLB**" for 1~3 mL input samples; select a program "**CFDNA-DLB**" for 4~6 mL input samples. The parameters are given in the following section.
6) Follow the guide shown on the screen and place plates carefully. Make sure that the chamfer of the plate faces toward the same direction.
7) Carefully remove the Plates when the program is finished.
8) Use micropipette to transfer the purified nucleic acids from **plate #6** to a clean tube.
9) Discard used Plates and spin tips into the waste recycling bin.

11. Program

■ **Maelstrom 2410 series (for 1~3 mL input samples)**

Program Name: CFDNA-SLB								
Plate	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Volume (µL)	7800	-	1000	1000	1000	100	100	-
Keep Temp.	-	-	-	-	-	-	-	-
Action	For. U/D	-	For. U/D	For. U/D	For. U/D	For.	For.	-
Name	LB	-	WB1	WB2	WB2	EB	-	TIP

Step	Plate	Temp. (°C)	Mixing (min)	Mixing (rpm)	Collect (sec)	Vapor (min)	Pause
1	1	Off	10	1000	60	0	Off
2	3	-	10	1000	60	0	Off
3	4	-	2	1500	30	0	Off
4	5	-	2	1500	30	10	Off
5	6	Off	8	1500	60	0	Off
6	5	-	0.2	1500	0	0	Off
8	6	0	0.2	1500	0	0	Off

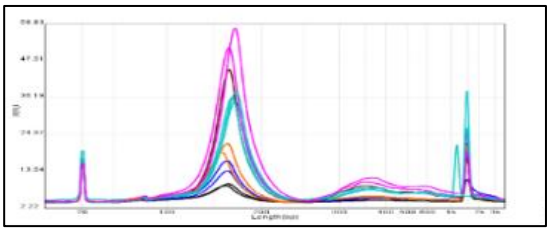
■ **Maelstrom 2400 series (for 4~6 mL input samples)**

Program Name: CFDNA-DLB								
Plate	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Volume (µL)	7800	7800	1000	1000	1000	100	100	-
Keep Temp.	-	-	-	-	-	-	-	-
Action	For. U/D	For. U/D	For. U/D	For. U/D	For. U/D	For.	For.	-
Name	LB	LB	WB1	WB2	WB2	EB	-	TIP

Step	Plate	Temp. (°C)	Mixing (min)	Mixing (rpm)	Collect (sec)	Vapor (min)	Pause
1	1	Off	10	1000	60	0	Off
2	2	-	10	1000	60	0	Off
3	3	-	10	1000	60	0	Off
4	4	-	2	1500	30	0	Off
5	5	-	2	1500	30	10	Off
6	6	Off	8	1500	60	0	Off
7	5	-	0.2	1500	0	0	Off

12. Result

Total DNA yield was quantified using Qubit dsDNA HS assay kit: 1~100 ng per mL serum / plasma. The expected peak bp (100~300 bp fragments) and low amount of gDNA contamination were observed by using Qsep 100 capillary gel electrophoresis system. The cfDNA isolate from 1-6 mL serum was shown below.



13. Reagent performance

■ **Repeatability**

Under repeatability conditions where nucleic acids are extracted with the same reagent kit on the same source samples by the same operator. The coefficient of variation of nucleic acid extraction concentration is less than 5%.

■ **Reproducibility**

A five-day reproducibility test was carried out with the same source samples for 5 consecutive days with the same reagent kit by different operators. The coefficient of variation of nucleic acid extraction concentration is less than 5%.

■ **The stability of extracted DNA**

Storage Conditions	DNAstability
-80°C	Over 90 days
-20°C	28 days
4°C	14 days
25°C	2 days
Freeze-thaw	5 times

14. Explanation of Symbols

	Manufacturer		**Consult instructions for use**
15°C 25°C	**Temperature limit**	Σ	**Contains sufficient for test**
CE	**CE mark**	IVD	***In vitro* diagnostic medical use**
REF	**Catalogue number**		**Caution**
LOT	**Batch code**	NON STERILE	**Non-sterile**
	Do not re-use		**Keep away from sunlight**
	Date of manufacture		**Use-by date**

EC REP

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15. Post-market surveillance conclusion

After a risk assessment and clinical evaluation assessment, when weighing the benefits of medical device, patients, and the risks associated with the use of the device, the risk is acceptable. The post-market surveillance report shows that no death or serious adverse events occurred.