Blood gDNA Extraction from Various Blood Specimen Types using GENTi™ Advanced Blood DNA Extraction Kit

Experimental Conditions

Materials Required

- GENTi™ Advanced Blood DNA Extraction Kit (903-048A/903-096A)
- GENTi™ 32 Advanced Automatic Extraction Equipment (GTI032A)
- Pipette & sterile pipette tips
- Suitable protector (e.g., lab coat, disposable gloves, goggles, and etc.)
- FTA card (for dried blood spot, DBS)

Sample Information

- Extraction conditions
  - Sample type and amount

Sample	Amount
Human whole blood	200 µl
Plasma	
Buffy coat	
DBS	2 dots

  - Elution volume: 80 ml

Sample Preparation

- **Human whole blood**

1. Collect 200 µl of whole blood in EDTA tubes or other anticoagulant mixtures.
2. Follow the **Protocol of GENTi™ Advanced Blood DNA Extraction Kit.**

- **Plasma**

1. Centrifuge whole blood at 3,000 x g for 15 minutes at 4˚C for blood to be separated into three components: plasma, buffy coat, and erythrocytes.
2. Pipette the plasma on the top layer and transfer it to new tube.
3. Follow the **Protocol of GENTi™ Advanced Blood DNA Extraction Kit.**

- **Buffy coat**

1. Centrifuge whole blood at 3,000 x g for 15 minutes at 4˚C for blood to be separated into three components: plasma, buffy coat, and erythrocytes.
2. Pipette buffy coat layer, being careful not to disturb the other blood components.
3. Follow the **Protocol of GENTi™ Advanced Blood DNA Extraction Kit.**

- **DBS**

1. Drop whole blood onto FTA cards (DBS).
2. Collect two 5 mm diameter DBS samples using sterilized punching machine.
3. Follow the **Protocol of GENTi™ Advanced Blood DNA Extraction Kit.**

Protocol

GENTi™ Advanced Blood DNA Extraction Kit Protocol

*** For more details, please refer to the manual of GENTi™ Advanced Blood DNA Extraction Kit.**

1. Peel off the foil seal from the pre-filled plate/tube.
2. Dispense 15 µl of dissolved proteinase K solution into the 1st (7th) well.
3. Transfer 200 µl or 2 dots of samples to 1st (7th) well.
4. Load the plate onto the tray of GENTi™ 32 Advanced System.
5. Insert Magnetic Rod Cover to the end of strip bracket.

Result

Sample	No.	Yield (µg)	$A_{260/280}$	$A_{260/230}$
Human whole blood	1	**5.31**	1.90	1.96
	2	**4.54**	1.81	1.88
	3	**4.49**	1.88	1.96
DBS	1	**0.17**	0.87	0.42
	2	**0.17**	1.05	0.46
	3	**0.18**	1.05	0.51
Buffy coat	1	**8.33**	1.86	2.16
	2	**6.57**	1.85	2.23
	3	**5.75**	1.86	2.13
Plasma	1	**0.77**	1.72	1.27
	2	**0.63**	1.81	1.53
	3	**0.66**	1.84	1.44

Figure 1. Result of spectrometer from four samples using GENTi™ Advanced Blood DNA Extraction Kit.
After DNA elutes were extracted from each samples with GENTi™ Advanced Blood DNA Extraction Kit, all DNA elutes were measured in triplicate with spectrometer such as yield, $A_{260/280}$, and $A_{260/230}$.
- **Spectrophotometer**
  NanoDrops 2000/2000C spectrometers (supplier T)

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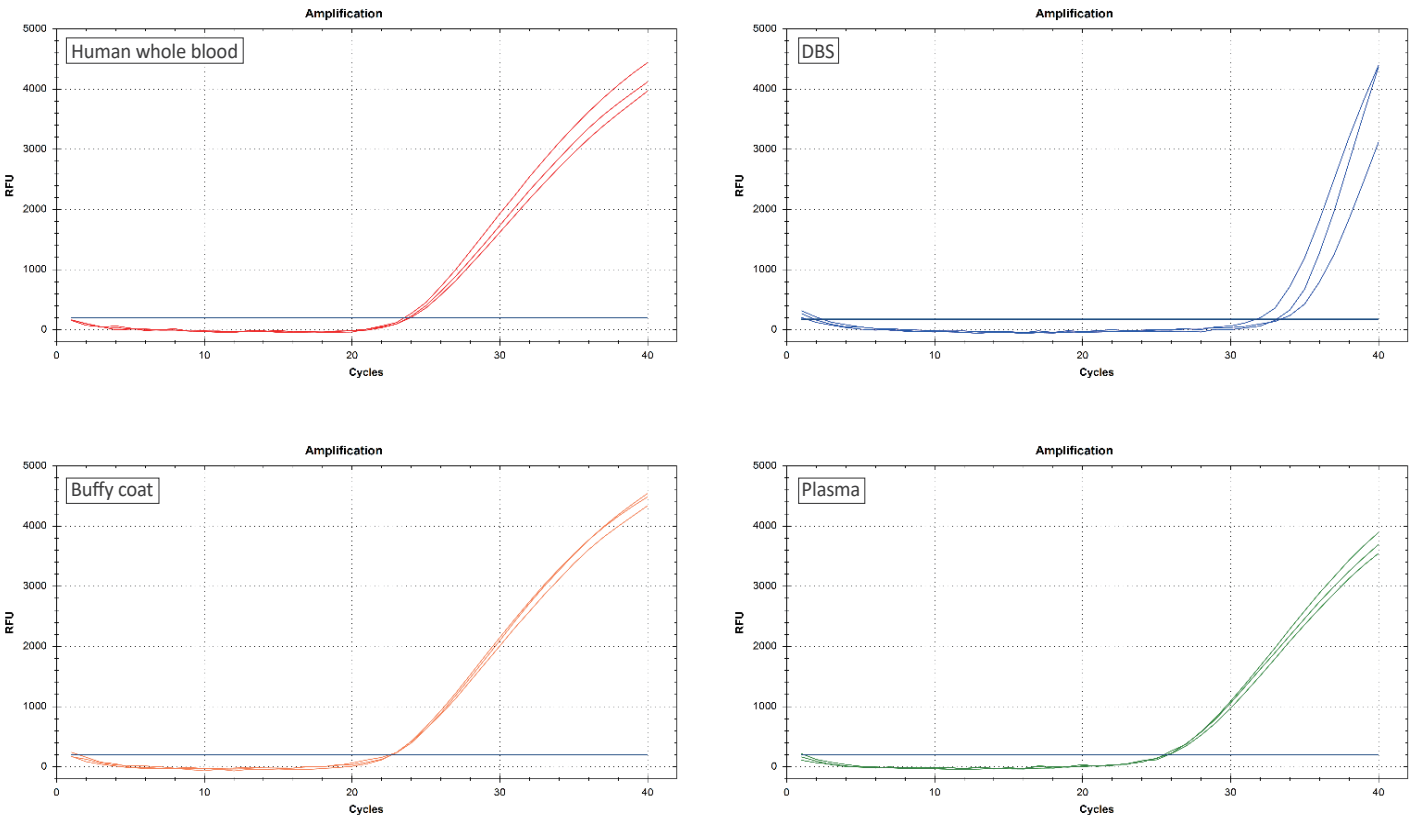
Result



Sample	Human whole blood			DBS			Buffy coat			Plasma		
No.	1	2	3	1	2	3	1	2	3	1	2	3
C_q Value	23.52	23.79	23.94	33.63	33.24	32.00	22.54	22.68	22.70	25.49	25.68	25.79

Figure 2. Result of real-time PCR from four samples using GENTi™ Advanced Blood DNA Extraction Kit.
After DNA templates were extracted from each samples with GENTi™ Advanced Blood DNA Extraction Kit, all DNA templates were performed in triplicate with real-time PCR methods.

- **PCR primer**
  Human GAPDH
- **Real-time PCR system and qPCR kit**
  Real-time PCR system: CFX-96™ System (1855201, supplier B)
  qPCR kit: Probe qPCR Mix (RR391AT)