TBG Biotechnology Corp.

EZbead™ Blood DNA Extraction Kit

Instructions for Use

For DNA isolation from whole blood

96 preps

REF 37100

REF 37100a

IVD

CE

Dec. 2017
Rev. 4.5

Intended use

The EZbead™ Blood DNA Extraction Kit is a magnetic beads base method intended for DNA isolation from blood.

Kit Contents

37100 User-filled reagent

Contents	Qty.
Lysis Buffer	50 ml
Magnetic Beads	6 x 1.5 ml
Wash Buffer 1	2 x 90 ml
Wash Buffer 2	20 ml
Wash Buffer 3	20 ml
Elution Buffer	20 ml
Deep Well Plate	6 pcs.
Mixing Rod	12 pcs.

37100a Pre-filled reagent

Contents	Qty.
Reaction Plate	6 pcs.
Mixing Rod	12 pcs.
Elution Buffer	20 ml

NOTICE:

1. 96 preps per kit.
2. All steps of this protocol should be performed at room temperature (20-30^{o}C) promptly.
3. Guanidine salt contained. Not compatible with disinfectant containing bleach.

Storage Conditions

All components of EZbead™ Blood DNA Extraction Kit can be stored dry at room temperature (20-30°C) for up to 1 year without showing any reduction in performance.

Other Materials Not Provided

- Micropipettes
- 96 – 100% Ethanol
- Heating Strips (Only for EZbead™ System 32 v1.0)
- Magnetic Block

Preparing Reagents for 37100 (user-fill) kits:

- Add 80 ml ethanol (96-100%) to Wash Buffer 2 before using.
- Add 80 ml ethanol (96-100%) to Wash Buffer 3 before using.

Important Notes

If the room temperature is below 20 °C, it is recommend to pre-heat lysis buffer, binding buffer, wash buffer 1 and elution buffer at 37°C for 10 min.

Protocol

Isolation of DNA from 200 µL whole Blood

1. Each deep well plate can be used to a perform 1~16 preps. For the EZbead™ System-32, a maximum of 2 plates can be used at once run totaling 32 tests.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1						Sample 9					
B	Sample 2						Sample 10					
C	Sample 3						Sample 11					
D	Sample 4						Sample 12					
E	Sample 5						Sample 13					
F	Sample 6						Sample 14					
G	Sample 7						Sample 15					
H	Sample 8						Sample 16					

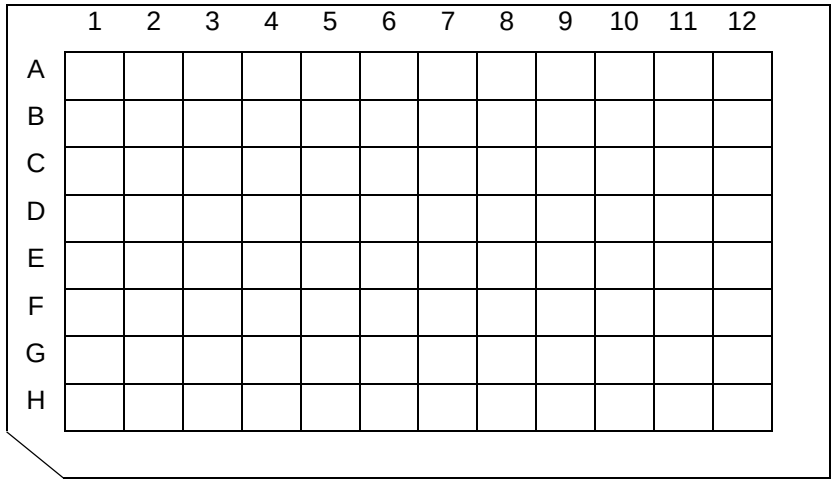
2. If you're using 37100a (pre-filled reagent kit), skip to step 3. If you're using 37100 (user-fill reagent kit), add the reagents for each well according to the following table.

Column No.	1 / 7	2 / 8	3 / 9	4 / 10	5 / 11	6 / 12
Reagent (Vol.)	Lysis Buffer (500 µL)	Wash Buffer 1 (800 µL)	Wash Buffer 1 (800 µL)	Mag. Beads (70 µL)* & Wash Buffer 2 (800 µL)	Wash Buffer 3 (800 µL)	Elution Buffer (130 µL)

*Vortex the magnetic beads before use.

3. Pipette 200µL of whole blood into wells on columns 1 or 7 of the deep well plate.

4. If you're using the EZbead™ system 16, simply slide the plate fully into the chamber. If you're using EZbead™ System-32, please place the plate over the heating strips underneath columns 6 and 12. In both EZbead™ systems, please ensure the bevel of the plate is positioned at the bottom left corner.



5. Slide the mixing rod fully into the EZbead™ System and close the cabin door.

6. Select the protocol "Blood-AUTO". Press "Start" to start the protocol run.

Setup of protocol “Blood-AUTO”:

For the EZbead™ System-32:

Well	Mix Min	Coll. Sec	Heat Min	Rod	Speed	Volume	Pause
4	1	60	0	On	M	800	Off
2	1	0	0	Off	M	800	Off
1	10	0	0	Off	L	500	Off
2	1	60	0	On	M	800	Off
1	10	60	0	On	M	500	Off
2	3	60	0	On	M	800	Off
3	3	60	0	On	M	800	Off
4	2	60	0	On	M	800	Off
5	2	60	0	On	M	800	Off
6	10	100	10	On	M	150	Off
5	1	0	0	Off	M	800	On

For the EZbead™ System-16:

Well	Mix Min	Coll. Sec	Rod	Speed	Volume	Pause	Vapor
4	1	60	On	M	800	Off	0
2	1	0	Off	M	800	Off	0
1	10	0	Off	L	500	Off	0
2	1	60	On	M	800	Off	0
1	10	60	On	M	500	Off	0
2	3	60	On	M	800	Off	0
3	3	60	On	M	800	Off	0
4	2	60	On	M	800	Off	0
5	2	60	On	M	800	Off	10
6	10	100	On	M	150	Off	0
5	1	0	Off	M	800	On	0

7. After the protocol run ends, press ”BZ stop” to stop buzzer and slide out the plate. Press “Start” again to return the robot arm to the starting position.

8. Place the plate on top of the magnetic block to magnetize any remnant magnetic beads to the bottom for 2 minutes. Transfer the elutant containing purified DNA in columns 6 and 12 to clean tubes for storage or continue to downstream applications.

WARNINGS AND PRECAUTIONS

1. Each laboratory has to perform the quality control test to ensure reliable results before running the specimen tests.

2. Please refer to the local legal requirements for waste management.

3. Please refer to the manufacturer‘s safety data sheet and the product labeling for information on potentially hazardous components. (MSDS could be obtained from local dealer.)

4. Do not use reagents past the expiration date printed on the label.

Troubleshooting

Issues	Suggestions
A_{260}/A_{280} ratio is low	1. Protein contamination: Decrease sample volume. 2. Increase homogenization time to ensure the sample is completely lysed. 3. Ensure washing steps are sufficient. 4. Repeat step 4 & 5 or increase the wash time.
A_{260}/A_{280} ratio is high	1. RNA contamination: Add RNase A (1 mg/ml) into lysate and incubate for 10 min at 37°C.
Low amount of extracted DNA	1. Adjust sample volume or amount of beads as necessary. 2. Extend elution time or conduct at 65 °C. 3. Incubation time of sample in lysis buffer can be extended. 4. Wash buffers which contain ethanol should be removed completely by elongating the heating time. 5. Magnetic beads should always be suspended after separation from the magnetic rods. 6. Double the elution time when water is used for elution.
Extracted DNA do not perform well in downstream application	1. Examine DNA concentration in the elutant and adjust the amount of elutant per test. 2. Check A_{260}/A_{280} ratio of purified DNA. 3. Wash buffers which contain ethanol should be removed completely by elongating the heating time.

EC REP

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