BIO BASIC
Quality-Affordable Research

Product information

QF 24 V4
V1 2020

Universal DNA Extraction Kit

Catalog #: WT3000
Size: 50 preps
Storage: 4°C

*: Product will be shipped at ambient temperature. Check storage conditions. Components have a one year expiration from time of purchase.

Product Description:

The kit is especially designed for Universal DNA Extraction. It simplifies the isolation of DNA from cell-free fluids with a fast spin-column format. No phenol/chloroform extraction is required. DNA binds specifically to the silica membrane while contaminants are removed in the flow-through. PCR inhibitors such as divalent cations and proteins are completely removed in two efficient wash steps, leaving pure DNA to be eluted in RNase-free water. Purified DNA is ready to use in RT-PCR, Northern blotting, or other downstream applications.

Features:

- **Fast:** Using a rapid spin column format, the entire procedure takes about 20 minutes.
- **High Yield:** The recovery yield of DNA is generally >85%.
- **Non-toxic:** No phenol/chloroform are used.

Material Supplied by User:

- Microcentrifuge capable of at least 12,000 × g
- DNase-Free pipets and pipet tips
- Vortexer
- DNase-Free Ethanol (96-100%)
- DNase-Free Microcentrifuge tubes (1.5 ml or 2 ml)

Before Starting:

i. Freshly Prepare the following ***Lysis Mastermix***:

Component	Volume per reaction	Volume per 50 reactions
Carrier DNA	2 µl	100 µl
Buffer Rlysis-VG	600 µl	30 ml

ii. Mix thoroughly by vortexing.

Composition:

Components	50 Prep Kit	250 Prep Kit	500 Prep Kit
Buffer Rlysis-VG	30 ml	150 ml	150 ml **x2**
Universal RPE Solution*	12 ml	30 ml **x2**	40 ml **x3**
Nuclease-Free Water**	5 ml	25 ml	50 ml
EZ-10 Spin Columns	50	250	500
2 ml Collection Tubes	50	250	500
Carrier RNA**	1 mg	1 mg	1 mg
Protocol	1		

***:** Universal RPE Solution is supplied in a concentrated form. Before use, add 48 ml 96-100% ethanol to 12ml concentrated universal RPE solution and mix well.

****:** Add Nuclease-Free Water to the tube containing lyophilized Carrier DNA to obtain a solution of 1 µg/µL. Dissolve the carrier DNA thoroughly, divide it into conveniently sized aliquots, and store it at -20°C. Do not freeze-thaw the aliquots of Carrier DNA more than 3 times.

Storage:

Components should be stored at room temperature. The kit is valid for 1 year at 4°C.

Procedure:

1. Sample preparation:

A) Dry samples:

Select between method **a.1** (without Proteinase K) OR **a.2** (with Proteinase K - optional):

a.1. Place the sample into a clean 1.5 ml microtube, and snap off the handle. Add 0.2 ml physiological saline, vortex for 30 seconds. Then transfer 0.2 ml solution to a new 1.5 ml microtube and proceed to step **2**.

a.2. If using Proteinase K (recommended for better yield):

i) Add 300 µl of PBS (user supplied) and 10ul of 20mg/ml Proteinase K solution (user supplied, *Bio Basic cat. #401*) to each swab sample.
ii) Incubate at 56°C for 5 minutes with occasional mixing.
iii) Centrifuge at 10,000 X g (or maximum speed) for 30 seconds
iv) Use 200 µl from step **3** as sample and proceed to step **2**.

B) Wet Samples:

I Vortex the tubes containing the sample at maximum speed for 1 minute
ii. Use 200 µl from step 1 as sample and proceed with step **2**.

***Note: **If transfer medium is very dilute i.e.** if the volume is **more than 400 µl**, **perform the below steps** for enrichment **instead of "i" and "ii":**
iii. Transfer appropriate liquid sample to a new 1.5 ml microtube,
iv. Centrifuge at 24,000 g for 60 minutes at 4°C. Then keep approx 0.2 ml solution in the tube but discard the remaining and continue to step **2**.

2. Add 0.6 ml of the recently prepared ***Lysis Mastermix*** into the tube (step **1**), vortex vigorously for 30 seconds; incubate at room temperature for 10 minutes.

NOTE: Lysis-Buffer-VG may form precipitate at 4°C, please dissolve it at 65°C and mix well before use.

3. Add equal volume of ethanol, mix by inverting the tube.

4. Transfer the mixture into the spin column; keep at room temperature for 2 minutes.

5. Spin at 10,000 g for 1 minute, discard the flow-through.

6. Add 0.5 ml of Universal RPE Solution***** to the column, spin at 10,000 g for 1 minute, and discard the flow-through.

***:** Universal RPE Solution is supplied in a concentrated form. Before use, add 48 ml 96-100% ethanol to 12ml concentrated universal RPE solution and mix well.

7. Repeat the Step **6** once.

8. Centrifuge at 10,000 g for 1 minute, discard the flow-through residue.

9. Transfer the column to a new 1.5 ml DNase-free microtube. Add 35-60 µl of Nuclease-Free Water onto the centre of the column; keep at room temperature for 2 minutes.

10. Spin at 10,000 g for 1 minute. Purified DNA is ready for use or keep at -20°C for long term storage.