Extraction of cfDNA from cancer plasma & cancer serum using Exgene™ cfDNA SV mini and detection of specific genes

Experimental Conditions

Materials Required

- Exgene™ cfDNA SV mini (129-101)
- cfDNA extraction commercial kit from Supplier A
- 1.5 ml microcentrifuge tube (DeNovo™, DMT150SA)
- Microcentrifuge (≤ 14,000 xg)
- Vortex mixer (DLAB, 8031102000)
- Pipette
- Sterilized pipette tips
- Suitable protector (e.g., lab coat, disposable gloves, goggles, etc.)

Sample Information

Sample type	Plasma or serum (derived from cancer patient)
Origin	Human
Target	cfDNA (cell-free DNA)

Extraction Conditions

Kit	Sample amount	Elution volume
Exgene™ cfDNA SV mini	300 µl	50 µl
Supplier A	500 µl	100 µl

* All elution steps used nuclease-free water, regardless of kit type.
* The extraction method followed each manufacturer's instructions.

Protocol

For more details and methods, please refer to the manual of Exgene™ cfDNA SV mini.

Preparation of Proteinase K

- **Proteinase K solution**

  Before starting the experiment, add 2.4 ml of PK-Storage buffer to lyophilized Proteinase K (48 mg) and mix carefully to avoid foaming.

Exgene™ cfDNA SV mini Protocol

1. Transfer 300 µl of sample to a 1.5 ml microcentrifuge tube (not provided).
2. Add 20 µl of Proteinase K solution (20 mg/ml, provided) and 300 µl of Buffer HL to the sample. Vortex vigorously to mix thoroughly for 30 sec and briefly spin down.
3. Add 300 µl of Buffer CL to the sample. Vortex vigorously to mix thoroughly for 30 sec and briefly spin down.
4. Incubate at 56 °C for 30 min and spin down briefly to remove any drops from inside of the lid.
5. Add 300 µl of chilled absolute ethanol (not provided) to the sample, pulse vortex to mix the sample thoroughly for 30 sec, and spin down briefly to remove any drops from inside of the lid.
6. Carefully transfer 650 µl of the mixture to the Column Type Q w/cap (mini), centrifuge at 13,000 rpm for 2 min at room temperature, discard the pass-through, and then reinsert the mini column back into the collection tube.
7. Repeat the step 6 using remained mixture.
8. Add 600 µl of Buffer HW1 to the column. Centrifuge at 13,000 rpm for 2 min at room temperature. Discard the pass-through, and then reinsert the mini column back into the collection tube.
9. Add 700 µl of Buffer HW2 to the column. Centrifuge at 13,000 rpm for 2 min at room temperature. Discard the pass-through and then insert the mini column into the new collection tube (provided).
10. Centrifuge at full speed for 3 min at room temperature to remove residual wash buffer. Place the mini column into a fresh 1.5 ml microcentrifuge tube (not provided).
11. Add 50 µl of nuclease-free water to the center of the membrane into the mini column. Incubate at room temperature for 2 min.
12. Centrifuge at full speed for 2 min at room temperature.

Result

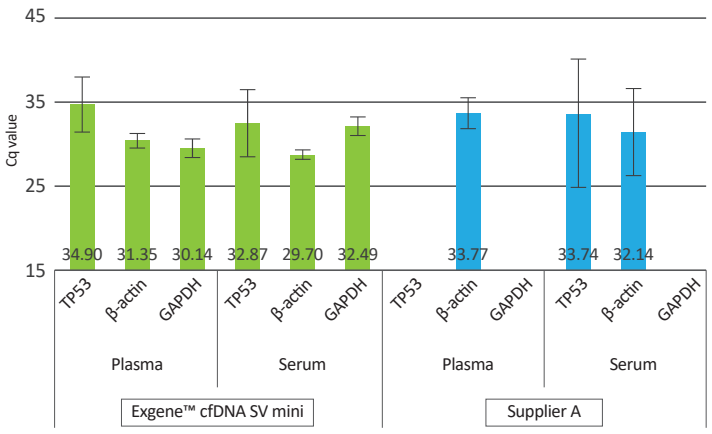

Table 1. Superior performance in real-time PCR.
cfDNA was extracted from plasma and serum from patients with a stage 3 gastric cancer using Exgene™ cfDNA SV mini and equivalent kits from Supplier A. The extracted cfDNA was used as a template for real-time PCR to detect three genes: human GAPDH, β-actin, and TP53. SYBR Green (human TP53, β-actin) and TaqMan Probe (human GAPDH) were employed for the detection.
- **qPCR system: CFX96™ System (1855201, Supplier B)**
- **qPCR kit (SYBR Green): RealAmp™ 2 X qPCR Mix (801-020)**
- **qPCR kit (TaqMan Probe): HyperScript™ One-step RT-PCR Master Mix (602-110)**