DNA extraction protocol for Cryptosporidium spps in stool samples (Adapted from GF-1 Soil Sample DNA extraction Kit)

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Abstract

The GF-1 Soil Sample DNA Extraction Kit is designed for the rapid and efficient purification of bacteria DNA from soil samples without the need for precipitation or organic extraction. The kit uses a high pure specially-treated silica-based material fixed into a column to efficiently bind DNA in the presence of high salt. The kit applies the principle of a minicolumn spin technology and the use of optimized buffers to ensure that only DNA is isolated while cellular protein, humic acid, metabolites, salts and other low molecular weight impurities are removed during the subsequent washing steps. We used this protocol for *Cryptosporidium* spp DNA extraction from stool samples with minor modifications and its working.

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Guidelines

All steps should be carried out at room temperature unless stated otherwise.

Before start

Pre-set the water bath at 70°C.

Materials

Centrifuges 5810 R View by Eppendorf Centrifuge

Micropipetes tips by Contributed by users

GF-1 Soil sample DNA extraction Kit GF-SD-025 by Vivantis Technologies Sdn. Bhd. Revongen Corporation Center No.12A, Jalan TP 5, Taman Perindustrian UEP, 47600 Subang Jaya, Selangor Darul Ehsan, Malaysia.

- Water bath at 70 oC by Contributed by users
- Micropippets (2-20 ul, 50-250ul, 100-1000ul) by Contributed by users
- Eppendorf tubes (1.5 & 2.0 ml) by Contributed by users
- Vertex mixer by Contributed by users

Protocol

Lysis

Step 1.

A. 1g of stool sample was taken in a 2ml microcentrifuge tube and 500mg (0.5g) of Glass beads was added. Than 1ml of SLX-buffer was added into each tube and vertexd at maximum speed for 5 minutes. Now, 100µl of DS-Buffer was added & vertexd agian for 3 minutes and then incubated at 70°C for 10 minutes.

B. The tubes were then Centrifugated at 5,000xg for 3 minutes at room temperature and 800µl of the supernatant was then moved into a new microcentrifuge tube. A 270µl of P2-Buffer was added into the sample tube and mixed thoroughly by vertexing and incubated on ice for 5 minutes. Then the tubes were Centrifugated again at 14,000xg for 3 minutes to pellet the stool sample and all the supernatant was again transferred into a new 2ml microcentrifuge tube.

AMOUNT
1 g : of stool sample
AMOUNT
500 mg : of Glass beads (provided in the Kit)
AMOUNT
1 ml : SLX-buffer
AMOUNT
100 µl : DS-Buffer
AMOUNT
800 µl : supernatant moved to new microcentrifuge tube
AMOUNT
270 µl : P2-Buffer was added and vertexed
DURATION
00:05:00 : vertex time at max speed
DURATION
00:03:00 : vertex time, again at max speed
DURATION
00:05:00 : vertex time (for ice incubation)

NOTES

During incubation mix the sample by vertexing twice.

DNA Precipitation

Step 2.

A 0.7 volume (700 µl) of iso-propanol was added and mixed well in the tubes by inverting the tubes 20-30 times and centrifugated at 14,000xg for 10 minutes. The supernatant was discarded carefully without dislodging the DNA pellet. The tubes were inverted for 1 min on the paper towel to drain the remaining liquid.

AMOUNT
700 µl : iso-propanol was added into tubes and mixed well
DURATION
00:10:00 : centrifugated at 14,000xg

DNA Solubilization

Step 3.

200µl of EB (Elution Buffer) was added to the tubes and assorted by pulse-vertexing and incubated at 70°C for 10-20 min to dissolve DNA pellet. 100µl of HTR Reagent was added and mixed thoroughly by vertexing for 10 secs and then incubated at room temperature for 2 mins. The tubes were again centrifuged at 14,000g for 2 mins and the supernatant was transferred into a new clean 1.5ml microcentrifuge tube.

AMOUNT
200 µl : Elution buffer added and mixed through pulse-vertexed
AMOUNT
100 µl : HTR-reagent added and mixed
TEMPERATURE
70 °C : incubation temp.
TEMPERATURE
20 °C : RT for incubation
DURATION
00:20:00 : for incubation
DURATION
00:00:10 : vertexing time for HTR reagent
DURATION
00:02:00 : for incubation

Protein digestion

Step 4.

2µl of Proteinase-K was poured to each tube (1.5 ml) and vortexed thoroughly and incubated at 37 °C for 10 mins. Then the XP1-Buffer equal to the sample volume (1.5 ml tube volume) was added and vortexed.

AMOUNT
2 µl : Proteinase-K was added and mixed

TEMPERATURE
37 °C : Incubation
DURATION
00:10:00 :

DNA wash and purification

Step 5.

A. The columns were inserted into each 2ml collection tube and 600µl of sample was transferred into each column. The tubes were centrifugated at 10,000 x g for 1 minute and the flow-through was discarded. Then 300µl of XP1 Buffer was added and centrifuged at 10,000 x *g* for 1 min and the flow thorough in the collection tubes was discarded.

B. The columns were inserted again into a new 2ml collection tube, and 700µl SPW Wash Buffer was added and centrifugated at 10,000 x *g* for 1 min, the flow thorough was discarded. The 700µl of SPW Wash Buffer step was repeated and the flow thorough was discarded.

C. The columns were centrifugated at 14,000g for 2 mins to remove all the traces of ethanol and then placed into a clean 2ml Eppendorf tube. 60µl of Elution Buffer was added directly onto the center of the membrane and incubated at 70°C for 5 mins.

AMOUNT
600 µl : sample was transferred to column
AMOUNT
300 µl : of XP-1 buffer was added and centrifugated at 10,000xg
AMOUNT
700 µl : SPW wash buffer was added into columns and centrifuged at 10,000xg
AMOUNT
60 µl : elution buffer was added onto the center on the cloumn membrane
TEMPERATURE
70 °C : Incubation of the columns
DURATION
00:01:00 : for centrifugation
DURATION
00:01:00 : Centrifuge time
DURATION
00:05:00 : incubation time

NOTES

The 700µl of SPW Wash Buffer step was repeated and the flow thorough was discarded.

Elution of DNA and storage

Step 6.

The columns were centrifugated at 14,000g for 1 min to elute DNA, and the elution phase was performed twice with a second 60µl of Elution Buffer. The columns were removed and discarded while the extracted DNA (Eppendorf tube) was stored at -20 ºC until amplification.

AMOUNT
60 µl : Elution buffer (repeated step)
TEMPERATURE
-20 °C : for DNA storage

Warnings

Buffer SB contains guanidine salts which can be harmful when in contact with skin or swallowed. Always wear gloves and practice standard safety precautions. Do NOT disinfect guanidine or extraction waste in solutions containing bleach or any other form of acid. To clean any items contaminated with the reagent, simply soak in detergent and water to remove all traces of guanidine before cleaning with bleach or acidic solution.