Presto™ Stool DNA Extraction Kit Quick Protocol

For research use only

Catalogue Number

STLD004, STLD050, STLD100

Instruction Manual Download

When using this product for the first time, or if you are unfamiliar with the procedure, please scan the QR code and download the complete instruction manual.

Instruction Manual Download

IMPORTANT BEFORE USE!

1. If precipitates have formed in ST1 Buffer, warm in a 37ºC water bath, followed by gentle shaking to dissolve.

2. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

1. Sample Lysis

Transfer **180-220 mg of stool to a Beadbeating Tube**. Very dry or fiber rich animal stool samples will absorb lysis buffer. In this case, reduce the stool amount to 60-80 mg. Human stool samples may contain undigested food, such as crop or fruit husks. These particles should not be transferred. Add **800 µl of ST1 Buffer**. Vortex briefly then incubate at 70ºC for 5 minutes. Attach the Beadbeating Tubes horizontally to a vortex with tape or adapter. Vortex at maximum speed for 10 minutes at room temperature. Centrifuge the at 8,000 x g for 2 minutes at room temperature to eliminate the foam caused by detergents present in ST1 Buffer. Transfer 500 µl of supernatant to a new 1.5 ml microcentrifuge tube.Preheat Elution Buffer (100 µl per sample) to 60ºC for DNA elution.

2. PCR Inhibitor Removal

Add **150 µl of ST2 Buffer** then vortex for 5 seconds. Incubate at 0-4ºC for 5 minutes. Centrifuge at 16,000 x g for 3 minutes at room temperature to precipitate insoluble particles and PCR inhibitors. Place an **Inhibitor Removal Column (purple ring) in a 2 ml Centrifuge Tube**. Transfer **500 µl of clear supernatant**. Centrifuge at 16,000 x g for 1 minute at room temperature then discard the column. Save the flow-through in the 2 ml Centrifuge Tube for DNA Binding. If a pellet is in the flow-through, transfer the clear supernatant to a new 1.5 ml microcentrifuge tube (not provided).

3. DNA Binding

Add **800 µl of SL3 Buffer** to the flow-through then mix IMMEDIATELY by shaking vigorously for 5 seconds. Place a **GD Column (green ring) in a 2 ml Collection Tube**. Transfer **700 µl of sample mixture** to the **GD Column**. Centrifuge at 16,000 x g for 1 minute at room temperature then discard the flow-through. Place the **GD Column** back in the **2 ml Collection Tube**. Transfer the remaining sample mixture to the **GD Column**. Centrifuge at 16,000 x g for 1 minute at room temperature. Discard the flow-through then place the **GD Column** back in the **2 ml Collection Tube**.

4. Wash

Add **400 µl of ST3 Buffer** to the **GD Column**. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the **GD Column** back in the **2 ml Collection Tube**. Add **600 µl of Wash Buffer (make sure absolute ethanol was added)** to the **GD Column**. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the **GD Column** back in the **2 ml Collection Tube**. Add **600 µl of Wash Buffer (make sure absolute ethanol was added)** to the **GD Column** again. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the **GD Column** back in the **2 ml Collection Tube**. Centrifuge at 16,000 x g for 3 minutes at room temperature to dry the column matrix.

5. Elution

Transfer the dry **GD Column** to a new 1.5 ml microcentrifuge tube. Add **30-100 µl of preheated Elution Buffer**[1], TE[2] or water[3] into the CENTER of the column matrix. Let stand for at least 2 minutes to allow **Elution Buffer**, TE or water to be completely absorbed. Centrifuge at 16,000 x g for 2 minutes at room temperature to elute the purified DNA.

[1]If a higher DNA concentration is required, use 30 µl of Elution Buffer (10 mM Tris-HCl, pH8.5) then repeat the Elution step by adding the same 30 µl of Elution Buffer (which now contains the eluted DNA) to the center of the column matrix again. If maximum DNA yield is required, use 100 µl of Elution Buffer (DNA concentration will be diluted). Ensure that Elution Buffer is added into the center of the GD Column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GD Column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the GD Column matrix and is completely absorbed. DNA eluted in water should be stored at -20ºC to avoid degradation.

Components

Component	STLD004	STLD050	STLD100
ST1 Buffer[1]	2 ml x 2	50 ml	85 ml
ST2 Buffer	1 ml	15 ml	30 ml
ST3 Buffer	10 ml	45 ml x 2	160 ml
Wash Buffer[2] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	25 ml (100 ml)
Elution Buffer	1 ml	6 ml	30 ml
Inhibitor Removal Column	4 pcs	50 pcs	100 pcs
GD Column	4 pcs	50 pcs	100 pcs
Beadbeating Tube (Type C)	4 pcs	50 pcs	100 pcs
2 ml Centrifuge Tube	4 pcs	50 pcs	100 pcs
2 ml Collection Tube	4 pcs	50 pcs	100 pcs

[1]If precipitates have formed in ST1 Buffer, warm the buffer in a 37ºC water bath, followed by gentle shaking to dissolve.

[2]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Storage

Dry at room temperature (15-25ºC)