Presto™ Mini Plasmid Kit Protocol

Protocol Procedure Without Color Indicator

1. Harvesting Transfer

1.5 ml of cultured bacterial cells (1-2 x 109 E. coli grown in LB medium) to a 1.5 ml microcentrifuge tube. Centrifuge at 14-16,000 x g for 1 minute at room temperature to form a cell pellet then discard the supernatant completely. Use a narrow pipette tip to ensure the supernatant is completely removed. Repeat the harvesting step as required for samples between 1.5-7.0 ml using the same 1.5 ml microcentrifuge tube.

NOTE: Using 2 OD600 - 6 OD600 units of bacterial culture is recommended. Do not use overgrown bacterial cultures (≤16 hours incubated in a culture tube at 37ºC with 150-180 rpm shaking). Use fresh bacterial cultures only. Solid medium and liquid medium (i.e. LB medium) should contain an antibiotic such as ampicillin.

2. Resuspension

Add 200 µl of PD1 Buffer (make sure RNase A was added) to the 1.5 ml microcentrifuge tube containing the cell pellet. Resuspend the cell pellet completely by vortex or pipette. Continue to vortex or pipette until all traces of the cell pellet have been dissolved.

3. Cell Lysis

Add 200 µl of PD2 Buffer to the resuspended sample then mix gently by inverting the tube 10 times. Close PD2 Buffer bottle immediately after use to avoid CO2 acidification. Do not vortex to avoid shearing the genomic DNA. Let stand at room temperature for at least 2 minutes to ensure the lysate is homogeneous. Do not exceed 5 minutes.

4. Neutralization

Add 300 µl of PD3 Buffer then mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. Centrifuge at 14-16,000 x g for 3 minutes at room temperature. If using >5 ml of bacterial cells, centrifuge at 16-20,000 x g for 5-8 minutes. During centrifugation, place a PDH Column in a 2 ml Collection Tube.

5. DNA Binding

Transfer all of the supernatant to the PDH Column. Use a narrow pipette tip to ensure the supernatant is completely transferred without disrupting the white precipitate. Centrifuge at 14-16,000 x g for 30 seconds at room temperature then discard the flow-through. Place the PDH Column back in the 2 ml Collection Tube.

6. Wash For Improved Downstream Sequencing Reactions

Add 400 µl of W1 Buffer into the PDH Column. Centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through then place the PDH Column back in the 2 ml Collection Tube. Proceed with Wash Buffer addition.

NOTE: W1 Buffer is essential for efficient sequencing reactions by removing nuclease contamination and should be added prior to Wash Buffer addition. If you are not performing sequencing reactions, W1 Buffer is not required. Proceed directly to Wash Buffer addition. For Standard Plasmid DNA Purification Add 600 µl of Wash Buffer (make sure absolute ethanol was added) into the PDH Column. Centrifuge at 14-16,000 x g for 30 seconds at room temperature. Discard the flow through then place the PDH Column back in the 2 ml Collection Tube. Centrifuge at 14-16,000 x g for 3 minutes at room temperature to dry the column matrix. Transfer the dried PDH Column to a new 1.5 ml microcentrifuge tube.

NOTE: Perform Wash Buffer steps twice for salt sensitive downstream applications.

7. Elution

Add 50 µl of Elution Buffer1 , TE2 or water3 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 14-16,000 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 PDH Column matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated Elution Buffer (60~70ºC).

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 PDH Column matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated TE (60~70ºC). 3 If using water for elution, ensure the water pH is ≥8.0. ddH2 O should be fresh as ambient CO2 can quickly cause acidification. Ensure that water is added into the center of the PDH Column matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated water (60~70ºC).