prepIT®•Q2A

prepIT•Q2A laboratory protocol for the preparation of genomic DNA from 100 µL of sample from ORAcollect•DX (OCD-100A).

prepIT•Q2A will enable a rapid, liquid-based removal of inhibitors found in oral samples collected with ORAcollect•Dx OCD-100A chemistry.

The following step-by-step protocol describes how to prepare genomic DNA from a 100 µL aliquot of sample for direct input into downstream applications.

Reagents included

- prepIT•Q2A (catalog #: PT•Q2A, includes: Reagent AG and Reagent ST)

Equipment and reagents required, not provided

- Heating plate at 75°C
- Proteinase K (> 30 mAU/mg activity)
  Proteinase K stock preparation:
  Prepare a 24 mg/mL stock solution by dissolving lyophilized Proteinase K in nuclease-free water.
  Store in aliquots at -20°C.
- Dilution reagent: 10 mM Tris (pH 7.5 – 8.0), nuclease-free water or similar.

Warning and precautions

Precaution: Use Reagent ST in a well-ventilated area. Keep container closed when not in use.
See MSDS at www.dnagenotek.com

Product use limitations

Use prepIT•Q2A only as directed in this product guide.

Procedure

Purification steps	Notes
1. Transfer a 100 µL aliquot of each ORAcollect saliva sample to a 0.2 mL PCR tube or a 96 well plate.	
2. Add 2 µL of a 24 mg/mL Proteinase K (PK) suspension and pipette up and down 3x using the same tip to ensure PK is fully dispensed. Mix thoroughly by pipetting up and down 5x using a pipette set at 80 µL.	• See Proteinase K stock preparation.
3. Heat the aliquots at 75°C for 20 minutes. Samples can remain uncovered during heating.	• This heat treatment is essential for effective PK treatment. Failure to adhere to these parameters will negatively impact performance on downstream assay.

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Purification steps	Notes
4. Add 10 µL of Reagent AG.	
5. Add 20 µL of Reagent ST and mix thoroughly by pipetting up and down 12x using a pipette set at 100 µL.	• Use in a well ventilated area, keep bottle closed when not in use. Reagent has a noticeable aroma. • Take caution not to get reagent on the surface-edge of the tube or plate-well, this will result in difficulty sealing the tube or plate.
6. Incubate the samples undisturbed at room temperature for 15 minutes. Alternatively, centrifuge at 2500 *x g* for 2 minutes.	• Incubation or centrifugation will result in the sample separating into two phases with the upper phase containing DNA.
7. Transfer 25 µL of the upper aqueous phase to a new tube or storage plate.	• Be careful not to disturb the bottom phase as it contains impurities. • DNA is fully prepared at this point. • Automated liquid handling is recommended for the 96 well plate format as the distinction between phases may be difficult to see in all wells.
8. Proceed directly to assay.	• A dilution may be required for optimal performance.
9. Prepared DNA can be stored at 4°C for up to 1 week.	• Ensure tube or plate are properly sealed to prevent evaporation.

Please visit our website at **www.dnagenotek.com** for a full page version of this protocol and any additional languages. Reference PD-PR-00840 for this protocol.

Technical support is available Monday to Friday (9h00 to 17h00 ET):

- Toll-free (North America): 1.866.813.6354, option 6
- All other countries: +1.613.723.5757, option 6
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