Quick to assay protocol handbook

for use with

prepIT®·Q2A

DNAgenotek™

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IVD

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prepIT•Q2A laboratory protocol

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Australian Sponsor:
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Label legend:

IVD	In vitro diagnostic medical device
REF	Catalog number
15°C 25°C	Storage instructions
	Manufacturer
LOT	Lot number

Patent (www.dnagenotek.com/legalnotices)

prepIT®•Q2A product overview

For preparation of genomic DNA from ORAcollect® and Oragene®

prepIT®•Q2A(PT-Q2A-96, PT-Q2A-384) will enable a rapid, liquid-based removal of inhibitors found in saliva samples collected with ORAcollect® and Oragene® devices.

The following step-by-step protocols describe how to prepare genomic DNA for direct input into downstream applications:

For preparation of genomic DNA from 100 µL of sample from ORAcollect (OCD-100, OCD-100A, OCR-100, OC-175) in 96-well plate format

PT-Q2A-96 reagents included

- Reagent AG (ref: PT-QAG-96)
- Reagent ST (ref: PT-QST-96)

PT-Q2A-384 reagents included

- Reagent AG (ref: PT-QAG-384)
- Reagent ST (ref: PT-QST-384)

Equipment and reagents required, not provided

- Heating block for a 96-well plate at 75°C
- 96-well PCR plate
- Dilution reagent: 10mM Tris (pH 7.5 – 8.0), nuclease-free water or similar (as required)
- Proteinase K (> 30 mAU/mg activity) **(required for OCD-100A preparation only)**

  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. 1 mL stock solution is sufficient for 500 sample preparations.
- 96-well storage plate

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 handbook. This protocol is intended to be performed by an automated liquid handler. Consult your DNA Genotek representative for a manual version of this protocol.

Procedure

Purification steps	Notes
1. Transfer a 100 µL aliquot of each ORAcollect sample to a 96-well plate.	
2. **This step applies to OCD-100A ONLY.** Proceed to Step 3 if preparing OCR-100, OC-175 or OCD-100 samples. Add 2 µL of a 24 mg/mL Proteinase K (PK) suspension. Mix by pipetting 5× with volume set at 80 µL.	• See Proteinase K stock preparation instructions on page 3.
3. Heat the plate at 75°C for 20 minutes.	• **This heat treatment is essential.** Failure to adhere to these parameters will negatively impact performance on downstream assay. • Samples can remain unsealed during heating.
4. Add 10 µL of Reagent AG.	
5. Add 20 µL of Reagent ST and mix thoroughly by pipetting 12× with volume set at 100 µL.	• Use in a well ventilated area, keep bottle closed when not in use. Reagent has a noticeable aroma. • Thorough mixing is required to ensure sufficient removal of impurities from the sample.
6. Incubate the samples undisturbed at room temperature for 15 minutes.	• A phase separation will occur in this step. The upper phase contains DNA. • This step may alternatively be performed manually by centrifuge at 2,500 × *g* for 2 minutes.
7. Transfer 25 µL of the upper phase to a 96-well storage plate.	• Be careful not to disturb the bottom phase as it contains impurities. • DNA is fully prepared at this point.
8. Proceed directly to assay.	• A dilution may be required for optimal assay performance. See suggested dilution reagents in the equipment and reagents section on page 3. • Samples are not suitable for DNA purity assessment by spectrophotometry due to reagent interference. • If DNA quantification is desired, quantification should be performed by a fluorescent assay, such as with PicoGreen® or SYBR® Green I.
9. Prepared DNA can be stored at 4°C for up to 1 week or at -20°C for long-term storage.	• Ensure tube or plate are properly sealed to prevent evaporation.

For preparation of genomic DNA from 100 µL of sample from Oragene (OGX-XXX) in 96 deepwell plate format

PT-Q2A-96 reagents included
- Reagent AG (ref: PT-QAG-96)
- Reagent ST (ref: PT-QST-96)

PT-Q2A-384 reagents included
- Reagent AG (ref: PT-QAG-384)
- Reagent ST (ref: PT-QST-384)

Equipment and reagents required, not provided

- Air or water incubator at 50°C
- 96 deepwell heating block at 75°C
- 96 deepwell plates (e.g., Abgene™ 1.2 mL round bottom, AB-0564)
- Vortexer at 1,300 rpm
- Dilution reagent: 10mM Tris (pH 7.5 – 8.0), nuclease-free water or similar (as required)
- 96-well storage plate

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 handbook. This protocol is intended to be performed by an automated liquid handler. Consult your DNA Genotek representative for a manual version of this protocol.

Procedure

Purification steps	Notes
1. Mix the sample by inverting the capped tube 5×.	• This is to ensure that viscous samples are properly mixed.
2. Incubate the sample at 50°C in a water incubator for a minimum of 1 hour or in an air incubator for a minimum of 2 hours.	• This heat-treatment step is essential to ensure that DNA is adequately released and that nucleases are permanently inactivated. • The entire sample must be incubated in the original collection tube before aliquoting to ensure sample homogeneity. • This incubation step may be performed at any time after sample is collected and before it is purified. • The sample may be incubated at 50°C overnight if it is more convenient. • A longer time is required in an air incubator because temperature equilibration is slower than in a water incubator.
3. Transfer a 100 µL aliquot of each Oragene sample to a 96 deepwell plate.	
4. Heat the 96 deepwell plate at 75°C for 10 minutes.	• **This heat treatment is essential.** Failure to adhere to these parameters will negatively impact performance on downstream assay. • Samples can remain unsealed during heating.
5. Add 10 µL of Reagent AG.	

Purification steps	Notes
6. Add 20 µL of Reagent ST and mix thoroughly by vortexing for 2 minutes at 1,300 rpm.	• Use in a well ventilated area, keep bottle closed when not in use. Reagent has a noticeable aroma. • Thorough mixing is required to ensure sufficient removal of impurities from the sample. • Vortexing can be performed unsealed with the 96 deepwell plate, however, vortexing above 1,300 rpm is not recommended to avoid spillage across wells. • If sealed plates are preferred, take caution not to get reagent on the surface-edge of the plate-well. This will result in insufficient plate sealing and cross-contamination while vortexing.
7. Incubate the samples undisturbed at room temperature for 15 minutes.	• A phase separation will occur in this step. The upper phase contains DNA. • This step may alternatively be performed manually by centrifuge at 2,500 × *g* for 2 minutes.
8. Transfer 25 µL of the upper phase to a 96-well storage plate.	• Be careful not to disturb the bottom phase as it contains impurities. • DNA is fully prepared at this point.
9. Proceed directly to assay.	• A dilution may be required for optimal assay performance. See suggested dilution reagents in the equipment and reagents section on page 6. • Samples are not suitable for DNA purity assessment by spectrophotometry due to reagent interference. • If DNA quantification is desired, quantification should be performed by a fluorescent assay, such as with PicoGreen or SYBR Green I.
10. Prepared DNA can be stored at 4°C for up to 1 week or at -20°C for long-term storage.	• Ensure plate is properly sealed to prevent evaporation.

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