OMNIgene™·XTRACT

Extraction of microbial DNA from fecal samples

REF **OM-XT-50**

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Purpose

The OMNIgene™•XTRACT extraction kit allows for the extraction of microbial DNA from fecal samples collected and stabilized in OMNIgene™•GUT (OMR-200, OM-200 and OMD-200) devices, as well as from unstabilized fecal samples.

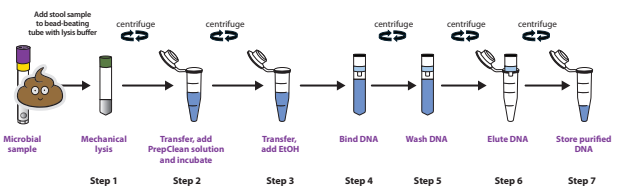
The OMNIgene™•XTRACT kit is intended for molecular biology applications and is for research use only. This kit is not intended for diagnostic applications.

Materials

Store all kit components and reagents at room temperature (15°C-30°C/59°F–86°F).

Component	Quantity
Lysis buffer	30 mL
PrepClean solution	19 mL
Bead-beating tubes	50 units (1 bag)
Silica columns	50 units (1 bag)
1.5 mL tubes	150 units (3 bags of 50 units)

Workflow overview



Protocol: Extraction of DNA from fecal microbiome samples

Supported sample types	
DNA Genotek™ collection device(s)	OMNIgene™•GUT (OMR-200, OM-200 and OMD-200)
Other sample types	Unstabilized feces

Equipment and reagents to be supplied by user

- Pipettors (20 µL-200 µL, 100 µL-1000 µL)
- Microcentrifuge (up to 14,000 x *g*)
- Vortex and vortex adapter for bead beating, compatible with microcentrifuge tubes
- 95%-100% ethanol
- 70% ethanol
- Nuclease-free water

Notes before starting

The lysis buffer is provided as a 2x concentrate. When using the kit for the first time, add 30 mL of nuclease-free water to the lysis buffer and mix by inversion.

Extraction procedure

1. Vortex the OMNIgene™•GUT collection tube at maximum speed for 10 seconds.
2. Transfer a 200 µL aliquot from the OMNIgene™•GUT tube to a bead-beating tube and add 800 µL lysis buffer.
   Notes:
   • If extracting unstabilized feces, transfer an amount equal to the size of a grain of rice (approximately 50 mg) to a bead-beating tube and add 1000 µL lysis buffer.
   • Adding an excess of feces (> 100 mg) may negatively impact extraction performance. A small amount of feces (~ 50 mg) will provide adequate yields.
3. Cap the bead-beating tube and secure it to the vortex adapter, with the cap oriented toward the center. Vortex for 15 minutes at maximum speed.
4. Centrifuge the bead-beating tube for 2 minutes at 14,000 x *g*. Transfer up to 600 µL of supernatant to a 1.5 mL tube without disturbing the pellet.
5. Add 300 µL PrepClean and vortex for 10 seconds at maximum speed.
6. Incubate tube for 5 minutes on ice and centrifuge for 2 minutes at 14,000 x *g*.
7. Transfer the entire volume of supernatant to a new 1.5 mL tube without disturbing the pellet.
8. Add 400 µL 95% ethanol and vortex briefly to mix.

9. Load 750 µL lysate onto a silica column and centrifuge for 1 minute at 14,000 x *g*. Discard flow-through and place the silica column back into the 2 mL collection tube.
   Note: Do not discard the 2 mL collection tube as it will be re-used for steps 10-12.
10. Repeat step 9 to process entire volume of lysate through the column.
11. Add 750 µL 70% ethanol to the silica column and centrifuge for 1 minute at 14,000 x *g*. Discard flow-through.
12. To fully dry the silica column, centrifuge for 1 minute at 14,000 x *g*.
13. Place the silica column in a new 1.5 mL tube.
14. Add 100 µL nuclease-free water to the center of the silica column membrane and incubate for 1 minute at room temperature.
15. Centrifuge for 1 minute at 14,000 x *g*. Discard the silica column and store eluted DNA at -20°C.

Post-extraction processing

Quantify DNA using a fluorescence-based assay, such as Quant-iT PicoGreen dsDNA Reagent (Thermo Fisher Scientific, Cat. No. P7589).
Note: Quantification of DNA using absorbance-based methods can result in inaccurate measurement of the yield.

Troubleshooting

Problem		Solution
Low DNA yields	**Sample-related**	1. Ensure that the sample was collected properly. Poor sample collection can negatively impact yields. For sample collection with DNA Genotek™ OMNIgene™•GUT (OMR-200, OM-200 and OMD-200) devices, follow the instructions provided. 2. Ensure that the sample is supported by the extraction protocol. (See supported sample types.) 3. Ensure that the sample was properly stored post-collection. Unstabilized fecal samples are prone to degradation and may result in low yields 4. Yields vary widely across donors and fecal types based on the Bristol scale. Some samples will naturally yield lower amounts of nucleic acids than others (i.e., infant fecal samples). Check if yields match what is typically expected for the sample type.
	Extraction-related	1. Ensure that an appropriate amount of sample was used as input into the extraction. 2. Ensure the step to dry the silica column was performed to remove residual ethanol prior to eluting nucleic acids. 3. When working with samples expected to contain lower biomass (i.e., infant fecal samples), lower the elution volume to increase DNA concentration.

Troubleshooting

Problem		Solution
DNA appears degraded	**Sample-related**	1. Ensure that the sample was collected properly. Poor sample collection can negatively impact nucleic acid quality. For sample collection with DNA Genotek™ OMNIgene™•GUT (OMR-200, OM-200 and OMD-200) devices, follow the instructions provided. 2. Ensure that the sample was properly stored post-collection. Samples stored without stabilization solution are prone to degradation.
	Extraction-related	1. Ensure that an appropriate amount of sample was used as input into extraction. Fecal matter contains high levels of nucleases and adding large amounts of fecal material (i.e., processing ≥ 100 mg of fecal matter) can lead to sample degradation. 2. Ensure that the recommended bead-beating conditions were used for lysis. Excessive bead beating can lead to shearing of DNA. 3. Contaminated reagents can introduce nucleases in samples. Ensure that a clean aliquot of nuclease-free water is used for elution.

Troubleshooting

Problem		Solution
Low purity/ presence of inhibitor	**Sample-related**	1. Ensure that the sample was collected properly in DNA Genotek™ OMNIgene™•GUT (OMR-200, OM-200 and OMD-200) devices (this does not apply to unstabilized). Collecting an excess amount of a sample can lead to carryover of inhibitors in the extracted material.
	Extraction-related	1. Ensure that an appropriate amount of sample was used as input into extraction. Fecal matter contains high levels of inhibitors and overloading (i.e., processing ≥ 100 mg of fecal material) can lead to carryover of inhibitors in the eluate. If samples contain higher than expected levels of inhibitors, repeat extraction with ~ 25 mg of fecal material. 2. When working with samples suspected of having higher amounts of inhibitors, perform PrepClean incubation on ice for a longer period of time (10 minutes). 3. Do not disturb the pellet or beads when transferring the lysate after bead beating and PrepClean treatment. If large amounts of floating debris are observed after the first centrifugation, before proceeding to the next step, invert the tube several times and centrifuge for an additional 2 minutes at 14,000 x *g*.

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Some DNA Genotek products may not be available in all geographic regions.

All DNA Genotek protocols, white papers and application notes are available in the support section of our website at www.dnagenotek.com.

Label legend:

REF	Catalog number
	Manufacturer
	Consult package insert
LOT	Lot number
	Use by
15°C – 30°C 59°F – 86°F	Storage instructions

Patent (www.dnagenotek.com/legalnotices)

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