Protocol Guide v 1.2

GALEAS™ Bead Xtract: Urine gDNA

For Extraction of Genomic DNA from DNA from urine

GALEAS™ Bead Xtract: Urine gDNA (96)
(Cat #PRE_GAL_BXG_96)

GALEAS™ nonacus

Revision History

Revision	Date	Revision Description
Version 1.2	November 2024	Updates to Kit Contents Quantity and to Reagent preparation table.

Intended use

This protocol is intended to provide guidance for using the GALEASTM Bead Xtract: Urine gDNA kit (Catalogue No. PRE_GAL_BXG_96). Using the GALEASTM Bead Xtract: Urine gDNA kit, high quality genomic DNA can be extracted from urine samples and is suitable for any downstream applications, including qPCR and Next Generation Sequencing. The kit provides enough reagents to perform 96 extractions.

Key Features

- Fast and simple protocol, which enables extraction of genomic DNA from urine in under 3 hours.
- The GALEAS™ Bead Xtract: Urine gDNA Kit can be processed manually or using automated platforms.
- The procedure eliminates the need for funnels and vacuum steps, providing hands-free operation in automated protocols.
- The unique formulation of the lysis buffer and magnetic beads allow complete automation of the extraction process with minimal user intervention.
- The high binding capacity of the beads allows for maximum recovery of DNA from the sample, with high concentration even when eluting in low volumes (50-100 µl).
- The system utilises the reversible nucleic acid-binding properties of paramagnetic beads to make processing DNA simple, clean and efficient.
- Configurable elution volumes between 50-100 µL.

Kit contents

GALEAS™ Bead Xtract: Urine gDNA (96) - (PRE_GAL_BXG_96)

Reagent	Quantity	Storage Conditions
gDNA Lysis Buffer	35 mL	15-25 °C
gDNA Wash Buffer 1	36 mL	15-25 °C
gDNA Wash Buffer 2	45 mL	15-25 °C
Elution Buffer	16 mL	15-25 °C
Proteinase K	46 mg	15-25 °C (store at -18 to -25 °C after reconstitution)
gDNA Magnetic Beads	3.5 mL	Refrigerated 2-8 °C

Additional Required Materials/Equipment (Not supplied)

- 100% ethanol (molecular biology grade).
- 100% isopropanol (molecular biology grade).
- Nuclease-free water (molecular biology grade).
- Magnetic separation device for 1.5/2.0 ml microcentrifuge tubes.
- Incubator or heat block capable of reaching and holding 70 °C temperature. If possible, a thermomixer is recommended to allow continuous vortexing whilst heating.
- Vortex mixer.
- Swinging bucket centrifuge capable of accommodating 50 ml conical tubes.
- 1.5 ml /2.0 ml microcentrifuge tubes compatible with magnetic separation device used.
- Pipettes and tips suitable for pipetting volumes outlines in the procedures. This would typically be 10-100 µL (or 20-200 µL) and a 100-1000 µL.
  -Optional: 1000 µL Wide bore (wide orifice) tips.
- For quantification of extracted gDNA: Qubit™ dsDNA High Sensitivity kit (ThermoFisher- Q32854). Qubit™ 4 Fluorometer (ThermoFisher-Q33238) is required to run these.

Storage and Stability

All GALEAS™ Bead Xtract: Urine gDNA Kit components are guaranteed for at least 6 months from the date of shipping when stored as follows: gDNA Magnetic Beads must be stored at 2 to 8 °C for long-term use. Lyophilised Proteinase K may be kept at room temperature (15-25 °C) for up to 12 months, but must be stored at -18 to -25 °C after initial reconstitution. Store all other components at room temperature (15-25 °C). During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve such deposits by warming the solution at 37 °C and gently shaking. Do not use the Bead Xtract Urine gDNA kit or any components past the expiry date stated.

Reagent Preparation

Before using the kit for the first time, some of the reagents require additives. See table 1 below for details on how to prepare the reagents. Proteinase K is supplied in lyophilised form and needs to be reconstituted in nuclease free water before use.

Table 1 Reagent dilutions required before starting. Add the listed amount of diluent to the entire bottle of reagent.

Kit/component	Diluent to be added	Volume to add (mL)
gDNA Wash Buffer 1	100% Ethanol (molecular biology grade)	54
gDNA Wash Buffer 2	100% Ethanol (molecular biology grade)	135
Proteinase K	Nuclease-free water (molecular biology grade)	2.3

For long term storage, Proteinase K should be kept at -20 °C after reconstitution and can be aliquoted for ease of use.

GALEAS™ Bead Xtract: Urine gDNA Kit: Extraction from Urine Pellets

Before starting:
Open the packaging of the urine collection device and ensure no leakage has occurred. Ensure the urine collection device has been stored for no longer than 28 days at room temperature. It is not recommended to store urine in the refrigerator (2-8 °C).

1. Prepare buffers according to the reagent preparation instructions in Table 1.
2. Ensure all reagents are equilibrated to room temperature, including Proteinase K and gDNA Magnetic Beads.
3. Set a heat block, water bath or thermomixer to 70°C.
4. Shake or vortex mix the gDNA Magnetic Beads to fully homogenise before use.
5. Prepare a fresh mastermix of 100% isopropanol and gDNA Magnetic Beads as instructed in the table below. If preparing a mastermix for more than 1 reaction, increase the amounts of the components by 5% to account for pipetting losses.

Component	Amount per sample (µL)	Amount for 8 samples +5% overage (µL)
Proteinase K	20	168
gDNA Lysis Buffer	300	2520
⚠ **WARNING: Proteinase K - gDNA Lysis Buffer mastermix should be used within 2 hours**		

Component	Amount per sample (µL)	Amount for 8 samples +5% overage (µL)
100% Isopropanol (molecular biology grade)	450	3780
gDNA Magnetic Beads	30	252

6. Prepare a fresh mastermix of 100% isopropanol and gDNA Magnetic Beads as instructed in the table below. If preparing a mastermix for more than 1 reaction, increase the amounts of the components by 5% to account for pipetting losses

⚠ **WARNING: Isopropanol - gDNA Magnetic Beads mastermix should be used within 2 hours**

Protocol

1. Centrifuge the tubes containing urine at 1,500 x g for 10 minutes.
2. Carefully discard the urine aqueous phase by slowly pouring it into a dedicated waste vessel and retain the cell pellet.
3. Add 320 µL of Proteinase K/gDNA Lysis Buffer mastermix to each sample. Carefully pipette up and down to ensure the sample is thoroughly mixed.

   NOTE: Some samples may be difficult to pipette. If necessary, vortex the sample to force resuspension. It is recommended to use wide-bore pipette tips.

4. Transfer up to 520 µL of the pellet mixture to a 1.5 or 2 mL tube.

   NOTE: Samples will generate cell pellets of different sizes, causing variability in total volume at this step. Only transfer up to a maximum of 520 µL of sample + lysis mix to the new tube.

5. Mix by vortexing for 30 seconds and incubate at 70 °C for 1 hour, with continuous mixing. If constant vortexing is not possible, perform additional vortex mixes of 30 seconds every 15 minutes of incubation.
   Once step 5 is complete, set the heat block, water bath or thermomixer to 60 °C (for use in step 19).
6. Add 480 µL of Isopropanol/gDNA Magnetic Beads mastermix to each sample.
7. Mix by vortexing for 30 seconds and incubate at room temperature for 5 minutes, with continuous mixing.
   If constant vortexing is not possible, perform a second vortex mix of 30 seconds after the first 2 and a half minutes of incubation.
8. Place the tube on a magnetic separation device for at least 5 minutes.
9. Remove and discard the supernatant, retaining the beads.
10. Remove the tube from the magnetic separation device and add 700 µL of gDNA Wash Buffer 1.
11. Vortex mix for 30 seconds and place on the magnetic rack for at least 1 minute or until the beads clear from the solution.
12. Remove and discard the supernatant, retaining the beads.
13. Remove the tube from the magnetic separation device and add 700 µL of gDNA Wash Buffer 2.
14. Vortex mix for 30 seconds and place on the magnetic rack for at least 1 minute or until the beads clear from the solution.
15. Remove and discard the supernatant, retaining the beads.
16. Repeat steps 13-15 for a total of 2 washes with gDNA Wash Buffer 2
17. Using a 10 µl pipette, remove any remaining supernatant and allow the beads to dry for 5 minutes at room temperature.
18. Add between 50 and 100µL of gDNA Elution Buffer, depending on the desired final elution volume.
19. Vortex mix for 30 seconds and incubate at 60 °C for 5 minutes.
20. Briefly centrifuge to collect the condensation from the cap and place the tube on the magnetic rack for 2 minutes or until the elution is clear of beads.
21. Avoiding the beads, transfer 50-100 µL eluate to a new tube for storage or downstream processing.

Quality control procedure for extracted gDNA

A mimimum yield of 100 ng is anticipated if using the input amounts from this protocol. Yields will vary depending on input volume, biological sample variability and collection and storage conditions. A fluorometric assay such as the Qubit™ dsDNA High Sensitivity kit (ThermoFisher-Q32854) is recommended. Use of UV-Vis spectrophotometry methods (e.g. Nanodrop) for quantifying nucleic acids is not recommended.

Support

Please speak to us if you require support by contacting support@nonacus.com

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