DNA: BioRobot® EZ1 and EZ1 Advanced XL Extraction

Sample types

DNA can be extracted from the following samples using this procedure:

- non-hair samples, such as blood, saliva, and contact samples
- hair (only validated using EZ1 Advanced XL)
- lysed samples containing sperm

Principle

Using the instrument, DNA is isolated from lysates in one step by binding to the surface of beads in the presence of a chaotropic salt. The beads are separated from the lysates using a magnet.

The DNA is then washed and eluted in either water or TE buffer.

Materials and equipment

This procedure uses the following materials and equipment:

- EZ1 DNA Investigator Kit: Contains all the reagents and materials necessary to extract and purify DNA on the BioRobot®, including TE buffer, G2 buffer, proteinase K, and reagent cartridges. The reagent cartridges contained in the EZ1 DNA Investigator Kit are critical reagents and require a QC check prior to use in casework.
- 1 M dithiothreitol (for preparation and QC, refer to *DNA: Organic Extraction*)
- EZ1 DNA Investigator protocol card
- BioRobot® EZ1 or EZ1 Advanced XL workstation
- heat block
- vortex
- pipettes and barrier pipette tips
- sterile water

G2 buffer preparation

Prior to sample preparation, the G2 buffer is diluted in sterile water at a 1:1 ratio.

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Non-hair sample preparation

For samples other than hair, the samples are prepared for extraction as follows.

Step	Action
1	To an EZ1 sample tube, add 190µl of diluted G2 buffer (290µl can be used for more absorbent samples), 10µl of Proteinase K, and the appropriate amount of sample.
2	Vortex the tube briefly and incubate at 56±1°C for 15 minutes. *Optional*: Incubate for an additional 5 minutes at 95±1°C.
3	Remove the substrate. NOTE: If the sample has been consumed, retain the substrate.

Hair sample preparation

Hair samples are prepared for extraction as follows.

Step	Action
1	To an EZ1 sample tube, add 180µl of diluted G2 buffer, 10µl of Proteinase K, 10µl of 1 M dithiothreitol, and the appropriate amount of sample.
2	Vortex the tube briefly and incubate at 56±1°C for at least six hours. Vortex the tube once or twice during incubation.
3	Add another 10µl of Proteinase K and 10µl of DTT solution and vortex for approximately 10 seconds. Incubate at 56±1°C for at least two hours or until the hair samples are completely dissolved.
4	If any solid material remains, transfer only the incubated solution to a new sample tube for extraction.

Samples containing sperm

For samples that contain sperm, refer to *DNA: Differential Extraction* for sample preparation.

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Set-up and operation of the BioRobot® EZ1 and EZ1 Advanced XL

The following procedure is followed to set-up and operate the BioRobot® EZ1 and EZ1 Advanced XL.

Step	Action
1	Insert the EZ1 or EZ1 Advanced XL DNA Investigator card completely into the card slot of the BioRobot®. The card typically remains in the card slot at all times. Switch on the instrument.
2	Press "Start" to display the protocols menu. For EZ1 Advanced XL, press "Esc."
3	Press "1".
4	Press "2" to elute in TE buffer. For EZ1, press "1" to elute in 50µl, "2" to elute in 100µl, or "3" to elute in 200µl. For EZ1 Advanced XL, press "1" to elute in 40µl, "2" to elute in 50µl, "3" to elute in 100µl, or "4" to elute in 200µl.
5	For EZ1, press any key to proceed through the text displayed in the LCD and start worktable setup. For EZ1 Advanced XL, press "ENT" to proceed through the text displayed in the LCD and start worktable setup.
6	Invert 1-14 reagent cartridges twice to mix the magnetic particles. Tap the cartridges to deposit the reagents to the bottom of their wells.
7	Load the reagent cartridges into the cartridge rack (after sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place).
8	Load 1-14 opened elution tubes into the first row of the tip rack.
9	Load 1-14 tip holders containing filter-tips into the second row of the tip rack.
10	Load 1-14 opened sample tubes containing digested samples into the fourth row of the tip rack.
11	Close the workstation door.
12	Press "Start"- the automated purification procedure takes 15-20 minutes.

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Set-up and operation of the BioRobot® EZ1 (continued)

Step	Action
13	When the protocol ends, the LCD displays "Protocol finished." Open the workstation door.
14	Remove the elution tubes containing the purified DNA.
15	Clean as below.
16	Turn off the BioRobot®.

Cleaning of BioRobot® EZ1

The following procedure is used to clean the BioRobot® after running a protocol.

Step	Action
1	Clean the piercing unit of the pipettor head by first wiping with a tissue moistened with water and then a tissue moistened with ethanol. For EZ1 Advanced XL, select "MAN" by pressing "2" in the main menu. NOTE: The piercing unit is sharp - use precaution.
2	The worktable and rack may also be cleaned with ethanol and then water.
3	For the EZ1 Advanced XL, UV decontamination may be performed for 20-60 minutes by following the display messages. Remove elution tubes prior to UV decontamination. Press "ENT" after cleaning.