

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

QIAGEN EZ1 Advanced XL DNA Extraction from Bloodstains and Other Casework Samples		
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QIAGEN EZ1 Advanced XL DNA Extraction from Bloodstains and Other Casework Samples

This extraction is applicable for exemplar samples and all casework evidence samples **EXCEPT** suspected semen samples.

If multiple EZ1 extractions will be performed at a time by one analyst, it is suggested that the incubation times for each set be staggered by about 1 hour to allow for sufficient processing time.

Follow all relevant processes in the [General Guidelines for Forensic Biology and DNA Casework procedure](#).

Follow all relevant processes in the [BEAST DNA Worksheet Setup Manual](#) for creating and adding to worksheets and [BEAST DNA Worksheet Processing Manual](#) for how to record all relevant information while processing the worksheets.

1 Procedure

- 1.1 Retrieve sample cuttings in 1.5mL tubes. Compare the label on the tubes to the worksheet and confirm that you have the correct samples.
- 1.2 Take each sample into your custody.
- 1.3 Obtain one 1.5mL tube for the extraction negative and label it with the associated extraction negative label.
- 1.4 Retrieve reagents for the Digestion Master Mix and record the lot numbers. Consult the Mixture Information table for the exact amount of Diluted ATL and Proteinase K needed to prepare the Master Mix.

Digestion master mix (per sample)		
Diluted ATL (μ L)	Proteinase K (Qiagen) (μ L)	Total
480	20	500

- 1.5 Add 500 μ L master mix to each sample and the negative control.
- 1.6 Vortex each sample tube briefly to mix.

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- 1.7 Incubate all samples in a Thermomixer set to 56°C (+/- 3°C) for 1.5-2 hours at 900rpm. Record the thermomixer and the temperature reading on the temperature probe.
- 1.8 Record the 'Incubation Run By' review task.
- 1.9 During the incubation, prepare the sample tubes and set up the EZ1 instrument:
- 1.9.1 Print three copies of the tube labels:
- Obtain tubes and label them:
 - Spin baskets and 1.5mL collection tubes
 - 2mL screw cap tubes labels
 - QIAGEN 1.5mL screw cap elution tubes
- 1.9.2 Load the EZ1 instrument with the EZ1 reagent cartridges, tips, and tip holders.
- 1.9.2.1 Remove both the tube rack and cartridge rack from the EZ1.
- 1.9.2.2 Obtain one reagent cartridge for each sample and extraction negative and record the lot number.
- NOTE:** More than one reagent lot may be used if there are not enough individual cartridges available for your full batch. Please record the additional lot number and samples it was used for in the Extraction Run By review task comments box.
- 1.9.2.3 Invert reagent cartridges twice to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottoms of their wells. Check that the magnetic particles are re-suspended. Also, visually inspect each reagent well. All wells except wells 9 and 11 must contain liquid.

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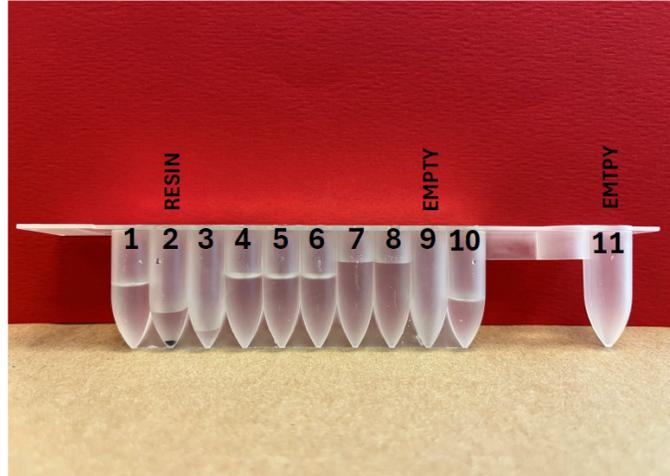
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EZ1/2 CARTRIDGES: Well volumes should appear as below:



Set aside any cartridges that do not appear to be filled like the above and alert QA.

- 1.9.2.4 Slide the EZ1 reagent cartridges into the cartridge rack. The cartridge label should be at the blunt end of the rack, closest to the tube rack when loaded onto the instrument. Place the rack in EZ1.



Reagent cartridge rack with cartridges loaded

- 1.9.2.5 Assemble the EZ1 tips and tip holders. Place them in row 2 of the EZ1 tube rack.
- 1.9.2.6 Record the EZ1 instrument.
- 1.10 Approximately 10 minutes before the incubation is completed, place the bottle of MTL buffer in the incubator to warm it to approximately 65°C.
- 1.11 Once the incubation is completed, briefly centrifuge the sample tubes to remove condensation from the tube caps.

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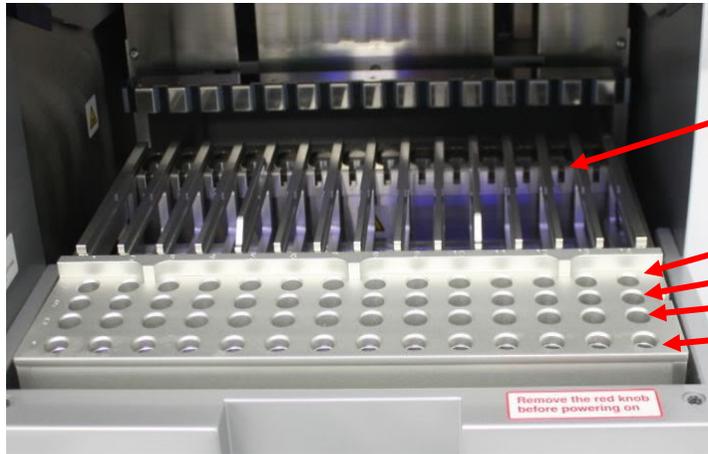
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- 1.12 **Tube Setup WITNESS:** Have another analyst verify three sets of tubes by reading the sample labels:
- Original incubation tube
 - Spin basket tube
 - 2mL screw cap tube
- 1.12.1 Record the 'Tube Setup Witness'.
- 1.13 Prepare 10% bleach, distilled water, and 70% alcohol in three 50mL conical tubes. Clean a pair of forceps by dipping the forceps in each of the three tubes briefly and then dry with a fresh lint free wipe.
- 1.14 For each sample, transfer the substrate using the forceps to its associated spin basket tube. Pipette mix the sample lysate within the incubation tube and then pipette the entire lysate volume (~500 μ L) over the top of the substrate in the spin basket. Close the tube top over the spin basket. Repeat this step for each sample, cleaning and drying the forceps between each sample.
- 1.15 Spin the substrates in spin baskets at 13,200 rpm for 2 minutes.
- 1.16 Using clean and dry forceps, remove and discard the spin baskets containing the swab remains taking care to avoid bubbles at the rim of the open tube. Clean and dry forceps between each sample.
- 1.17 Transfer the lysate from the spin basket tube to the associated 2mL screw cap tube.
- 1.18 Remove the warmed MTL from the incubator and add 400 μ L of the warmed MTL buffer and 1 μ L carrier RNA to each 2mL sample tube, pipette mixing as needed, to ensure mixing of the MTL with the lysate.
- 1.19 To avoid possible precipitation, proceed directly with EZ1 DNA extraction protocol while the sample lysates are still warm.
- 1.20 **Instrument Setup WITNESS:** Have another analyst verify the instrument setup and loading of all samples and elution tubes onto the EZ1.
- 1.20.1 Load the labeled sample tubes into row 4 of the EZ1 tube rack, reading the label and discarding the screw cap as you load each sample.
- 1.20.2 Load the labeled elution tubes into row 1 of the EZ1 tube rack, reading the label and removing and retaining the screw cap as you load each sample.
- 1.20.3 Ensure the tube rack is loaded onto the instrument after the cartridge rack.

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- 1.20.4 Verify that all samples, reagents, and racks are loaded appropriately on the instrument.
- 1.20.5 Record the 'Instrument Setup Witness'.



Reagent cartridge rack – place into instrument **first**

Tube rack:

Row 4 – sample tubes

Row 3 – empty

Row 2 – pipette tips

Row 1 – elution tubes

EZ1 Worktable

- 1.21 Run the EZ1 protocol for purification of the samples:
 - 1.21.1 If needed, press 'ESC' to get to the main menu.
 - 1.21.2 From the main menu press 'Start' to begin a run.
 - 1.21.3 When asked if you would like to create a run report, press 'ESC' to select no.
 - 1.21.4 Press '3' to select the large volume protocol.
 - 1.21.5 Press '2' to select elution in TE.
 - 1.21.6 Press '1' to select the 40 μ L elution volume.
 - 1.21.7 The screen will display instructions to ensure the EZ1 has been loaded properly.
 - 1.21.8 After checking each step, press 'ENT' until the final step, and then press 'Start'.
 - 1.21.9 The protocol run time is ~18 minutes.
- 1.22 Create a reference tube with 36 μ L of 0.1X TE⁻⁴.
- 1.23 After the protocol is completed, press 'ENT' to continue.

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- 1.24 Open the instrument door and remove the tube rack. Remove the 1.5mL elution tubes, capping each sample.
- 1.25 Compare each extract to the reference tube containing 36µL of 0.1X TE⁻⁴. If the volume appears lower than 36µL, measure the extract.
 - 1.25.1 Measured volumes below 36µL should be documented in the Pre/Post Issue Log and in the sample comments field on the worksheet.
 - 1.25.2 Samples with measured volumes below 27µL must not proceed for further testing.
- 1.26 Record the 'Extraction Run By' review task and 'Response' dropdown for the 'Pass or Fail?' step.
- 1.27 Transfer custody of all extracts to a cryobox and store in a refrigerator or freezer.
- 1.28 Assign samples to next process step.

2 Clean the EZ1 instrument

- 2.1 DO NOT USE BLEACH ON THE INSTRUMENT, ONLY 70% ETOH WITH LINT FREE WIPES. NEVER SPRAY ETHANOL DIRECTLY ON THE INSTRUMENT.
- 2.2 Remove reagent cartridges, lysate tubes, tip-holders and tips, and any waste and discard appropriately.
- 2.3 Wipe down the inside of the instrument using a lint free wipe and 70% EtOH.
- 2.4 Close the EZ1 door.
- 2.5 Follow the prompts on the screen to start a UV run, setting the time to 20 minutes.
- 2.6 Note: The UV lamps need a minimum switch-on time of 20 minutes. Do not interrupt a UV light cycle before 20 minutes have passed since it will reduce the lamp's lifetime. Do not touch UV lamp with your fingers. Notify QA when a UV lamp needs to be replaced.