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. Refer to the Evidence Examination manual to determine the appropriate sample size to submit for extraction.

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.

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1 Procedure

1.1 Obtain labeled cuttings in 1.5 mL tubes. Compare your LIMS labels to the sample list on the DNA worksheet in LIMS and confirm that you have the correct samples. The maximum number of samples per batch is 14 (13 samples + 1 eneg).

1.2 Obtain one 1.5 mL tube for your extraction negative, and label it with the associated LIMS label.

1.3 Scan each sample into your custody.

1.4 Retrieve reagents for cell digestion and record the lot numbers in LIMS.

1.5 The Mixture Information table contains the reagent amounts for the master mix. Click **Recalculate** if you updated the number of samples on the worksheet and need to recalculate the amount. Use the volumes listed in the "Total Quantity" column to prepare the master mix (LIMS will calculate 1.2x the number of samples).

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

1.6 Add 500µl of master mix to each sample and the negative control.

1.7 Vortex each sample tube briefly to mix.

1.8 Incubate all samples at 56°C for 1.5-2 hours at 900rpm using a thermomixer. Record the thermomixer temperature and the instrument in the 'Additional Information' section. Add the instrument to the 'Instrument' box at the top of the worksheet..

1.9 During the incubation:

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1.9.1 Prepare your sample tubes:

1.9.1.1 Print your labels. For the entire protocol you will need:

- Three copies of the LIMS labels

1.9.1.2 Obtain your tubes and label them:

- Spin baskets and 1.5 mL collection tubes – **LIMS** labels
- 2 mL screw cap tubes – **LIMS** labels
- QIAGEN 1.5 mL screw cap elution tubes – **LIMS** labels

1.9.2 Load the EZ1 instrument with the appropriate reagent strips and tips.

1.9.2.1 Add the EZ1 instrument to the 'Instrument' box.

1.9.2.2 Remove both the tube rack and cartridge rack from the EZ1.

1.9.2.3 Obtain reagent cartridges and record the lot number in LIMS.

Note: More than one reagent lot may be used if there are not enough individual cartridges available for your full batch. Please indicate in the Extraction Run By review task comments box the additional lot number and for which samples it was used.

1.9.2.4 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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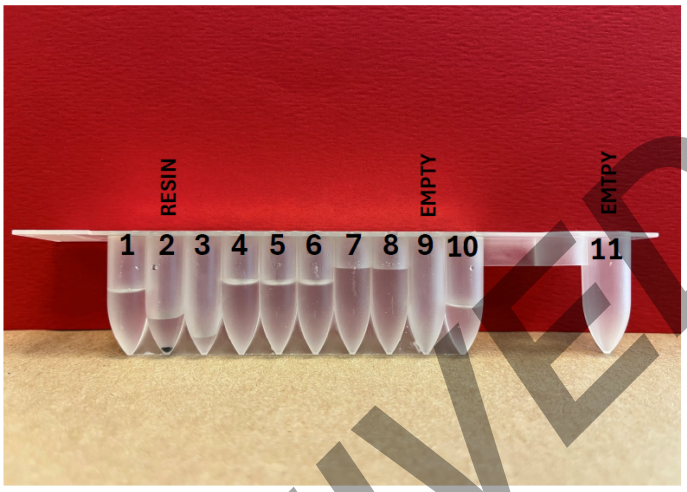
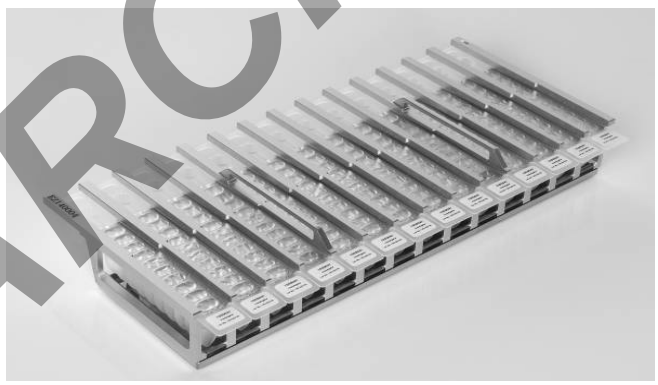
EZ1/2 CARTRIDGES: Well volumes should appear as below:



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

1.9.2.5 Slide the EZ1 reagent cartridges into the cartridge rack, one cartridge for each sample being run. The cartridge label side should be at the blunt end of the rack, closest to the tube rack when loaded in to the instrument. Place rack in EZ1.



Reagent cartridge rack with cartridges loaded

1.9.2.6 Assemble EZ1 tips and tip holders. Place them in row 2 of the EZ1 tube rack.

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.

1.12 Fill out the Incubation Run By Review.

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1.13 **Tube Setup WITNESS:** Have a witness verify three sets of tubes:

1.13.1 Original incubation tube (**LIMS** labels)

1.13.2 Spin basket tube (**LIMS** labels)

1.13.3 2 mL screw cap tube (**LIMS** labels)

1.14 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 drying with a fresh lint free wipe.

1.15 For each sample, transfer the substrate using the forceps to its associated spin basket tube. Clean and dry forceps between each sample

1.16 Pipette mix the sample lysate within the incubation tube and then pipette the entire lysate volume (~500µl) 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.

1.17 Spin the substrates in spin baskets at 13,200 rpm for 2 minutes.

1.18 Remove and discard the spin baskets (including the swab/stain remains) using forceps, taking care to avoid bubbles at the rim of the open tube. Clean and dry forceps between each sample.

1.19 Transfer the lysate from the spin basket tube to the associated 2 mL screw cap tube.

1.20 Remove the warmed MTL from the incubator and add 400µL of the warmed MTL buffer and 1µl carrier RNA to each 2 mL sample tube, pipette mixing as needed, to ensure mixing of the MTL with the lysate.

1.21 To avoid possible precipitation, proceed directly with EZ1 DNA extraction protocol while the sample lysates are still warm.

1.22 **Instrument Setup WITNESS**: Have a witness verify the lysates, elution tubes, and loading of the Extraction Negative and samples on to the EZ1.

1.22.1 Remove and discard the cap and load the sample tubes into row 4 of the EZ1 tube rack, reading the LIMS label.

1.22.2 Next, load the labeled elution tubes to row 1 of the EZ1 tube rack, reading the LIMS label and removing the screw cap as you load each sample.

1.22.3 Place tube rack into the EZ1. Make sure that the tube rack is loaded onto the instrument after the cartridge rack.

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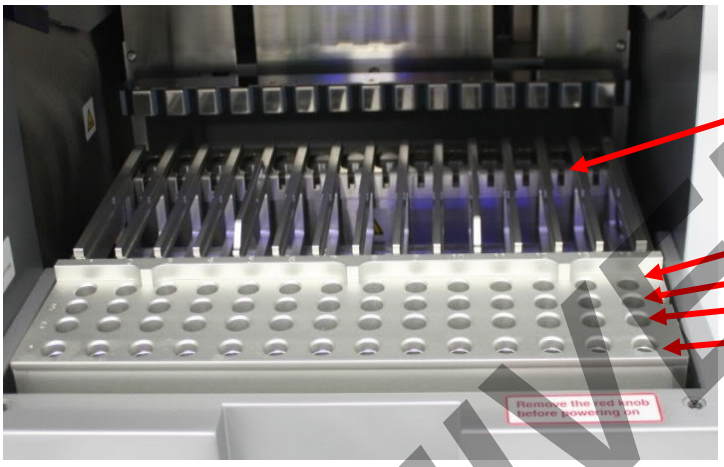
1.22.4 Witness should verify that all samples, reagents, and racks are loaded appropriately on the instrument.



EZ1 Worktable

1.23 Run the EZ1 protocol for purification of the samples:

1.23.1 If needed, press “ESC” to get to the main menu.

1.23.2 From the main menu press “Start” to begin a run.

1.23.3 When asked if you would like to create a run report, press “ESC” to select no.

1.23.4 Press “3” to select the large volume protocol.

1.23.5 Press “2” to select elution in TE.

1.23.6 Press “1” to select the 40µl elution volume.

1.23.7 The screen will display instructions to ensure the EZ1 has been loaded properly.

1.23.8 After checking each step, press “ENT” until the final step, and then press “Start.”

1.23.9 The protocol run time is ~18 minutes.

1.24 Create a reference tube with 36µl of 0.1X TE^{-4}.

1.25 After the protocol is completed, press “ENT” to continue.

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1.26 Open the instrument door and remove the tube rack. Remove the 1.5mL elution tubes, capping each sample with the labeled tube top.

1.27 Compare each extract to the reference tube containing 36µl of 0.1X TE^{-4}. If the volume appears lower than 36µl, measure the extract.

1.27.1 Measured volumes below 36µl should be documented in the Lab Supervisor Issues Log and the sample comments.

1.27.2 Samples with measured volumes below 27µl must not proceed for further testing.

1.28 Discard all the used cartridges, lysate tubes, tip-holders and tips.

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 and any waste; 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.

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.

2.6 Fill out the 'Response' dropdown and complete the Extracted Run By task.

2.7 Create the QUANTTRIO submission for the samples.

2.8 Verify the samples listed on the Trio Submission Worksheet click [Save] and close the Submission worksheet.

2.9 Store the extracts at 2°C to 8°C or frozen and record the transfer in LIMS.

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