July 2023

Important Note

EZ2® AllPrep® DNA/RNA FFPE Kit and EZ1&2™ ccfDNA Kit

Dear valued EZ2 instrument customer,

This note is to share with you an important piece of information that you should consider when working with your EZ2 instrument:

With the release of new Protocol Package v8, we are happy to give you more flexibility in choice of elution volumes for the EZ2 AllPrep DNA/RNA FFPE Kit (Table 1) and the EZ1&2 ccfDNA Kit (Table 2). Additionally, urine as new starting material, can now be processed as described below in the section *Processing urine samples.*

Table 1. EZ2 AllPrep DNA/RNA FFPE elution volume options

	RNA elution volume (µL)	DNA elution volume (µL)	Notes
Option 1	55	100	
Option 2	35	60	Yield from DNA-rich samples might be reduced because of limited binding capacity; please use DNA elution 100 µL for DNA-rich samples.
Option 3	35	40	Yield from DNA-rich samples might be reduced because of limited binding capacity; please use DNA elution 100 µL for DNA-rich samples.

Table 2. EZ1&2 ccfDNA sample type, sample input and elution volume options

Sample input volume	DNA elution volume (µL)
2, 4, or 8 mL (plasma or serum)	75
2, 4, or 8 mL (plasma or serum)	45
2, 4, or 8 mL (stabilized urine)	45

Stabilized human urine

Due to rapid degradation of circulating cell-free DNA after urine collection, it is strongly recommended to stabilize urine samples immediately.

After stabilization using a commercial solution (additives such as EDTA alone do not stabilize the cfDNA in urine sufficiently), it is recommended to centrifuge urine samples at low speed (1900 x *g*) for 10 minutes at room temperature (15–25°C) to remove cells prior to extraction of circulating cell-free DNA.

Non-stabilized human urine

We do not provide a ready-to-use stabilizing solution but recommend the following pre-treatment:

Before starting a protocol that requires Buffer ATL, check whether precipitate has formed in Buffer ATL. If necessary, dissolve by heating at 70°C with gentle agitation in a water bath. Aspirate bubbles from the surface of Buffer ATL.

It is recommended to centrifuge urine samples immediately after collection at low speed (1900 x *g*) for 10 minutes at room temperature (15–25°C) to remove cells. Non-stabilized urine samples require sample pretreatment.

Important: Equilibrate samples to room temperature (15–25°C) before starting pretreatment.

Important: Centrifugation and pretreatment should be performed within 4 hours of urine sample collection.

Mix 1800 µL urine or 3600 µL urine with 200 µL or 400 µL Buffer ATL, respectively. Incubate the samples at room temperature (15–25°C) for 1 hour.

Centrifuge samples at 1900 x *g* for 10 minutes at room temperature (15–25°C).

Processing urine samples

If precipitates are visible in the stabilized urine (e.g. after thawing), warm the samples to 25°C in a water bath to dissolve them.

Stabilized urine as sample input can be used directly with the EZ1&2 ccfDNA Kit the same as serum or plasma samples. Non-stabilized human urine samples should be pretreated as described. After transferring the supernatant to the large volume tube, please follow the "Purification of ccfDNA from up to 8 ml Serum or Plasma Using the EZ1&2 ccfDNA Kit" protocol of the kit handbook (**www.qiagen.com/HB-2915**), and select the protocol for urine on the EZ2 Connect device.

Important: Stability and integrity of circulating cell-free DNA is limited in non-stabilized urine; work quickly during ccfDNA extraction.

Note: Buffer ATL is not part of EZ1&2 ccfDNA Kit and it has to be ordered separately. Ordering information can be found at the Buffer ATL webpage(**www.qiagen.com/Buffer-ATL**).

For detailed instructions on how to use the EZ2 AllPrep DNA/RNA FFPE Kit and EZ1&2 ccfDNA Kit, refer to the handbooks, which can be found on their respective webpages (**www.qiagen.com/ez2-allprep-dna-rna-ffpe-kit** and **www.qiagen.com/ez1-and-2-ccfdna-kit**).

In case you have any questions, please reach out to QIAGEN Technical Services or your local QIAGEN representative.

Best regards,

QIAGEN