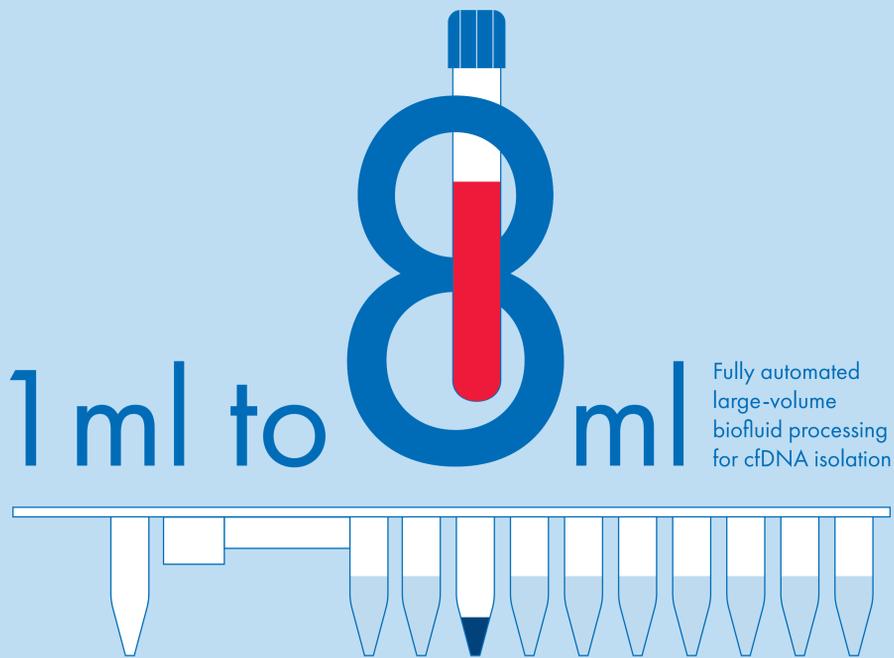


# Processing of large-volume liquid biopsies: cfDNA isolation and CTC enrichment with subsequent mRNA isolation



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## Liquid biopsy analysis made easy

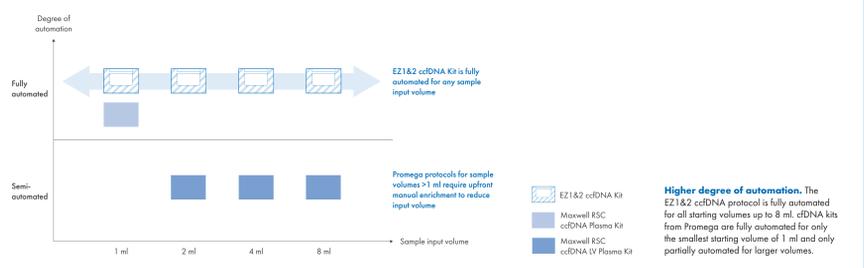


Liquid biopsy in cancer research – non-invasive sampling combined with powerful biomarker analysis – provides great potential for future use in diagnosis, therapy stratification, monitoring or early detection of cancer. But especially the low abundance of tumor DNA and cells can make it challenging to isolate cell-free DNA (cfDNA) and mRNA from circulating tumor cells (CTCs).

To cope with these challenges, we have developed protocols on the EZ2 Connect instrument for cfDNA isolation, as well as CTC enrichment and subsequent isolation of mRNA from these cells. Automation and standardization of these processes simplifies liquid biopsy processing.

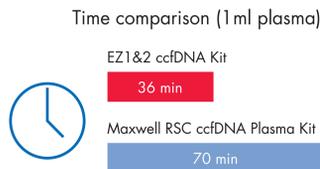
### Easy, fully automated processing of cfDNA samples

We designed the EZ1&2 cfDNA Kit protocol to be easy to use and fully automated for 1 to 8 ml starting volumes. We compared it with the protocols for cfDNA kits from Promega. The degree of automation provided by the EZ1&2 Kit was higher for 2, 4 and 8 ml starting volumes.



### Faster processing

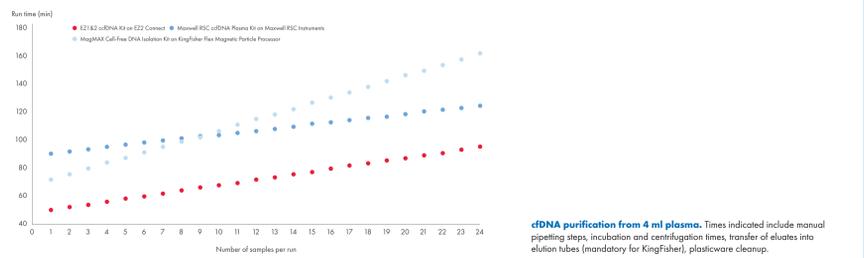
Short time to result is often critical. Therefore we compared run times. The EZ1&2 cfDNA Kit provides faster processing than the Maxwell RSC cfDNA Plasma Kit from Promega.



**Faster processing.** Run times for the EZ1&2 cfDNA Kit run on the EZ2 Connect and Maxwell RSC cfDNA Plasma Kit and the Maxwell system from Promega. Data are shown for processing 1 ml plasma sample.

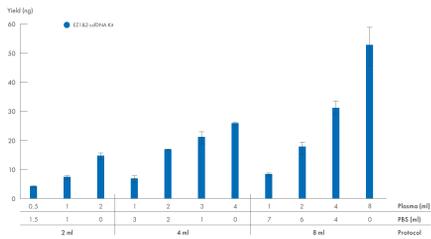
### Faster for any number of samples

Higher processing speed results in faster protocols and saves you time. We find this important and so compared run times for three alternative cfDNA isolation systems. The EZ1&2 cfDNA Kit provides faster processing than alternative systems from Promega and Thermo Fisher.



### Flexible starting volumes

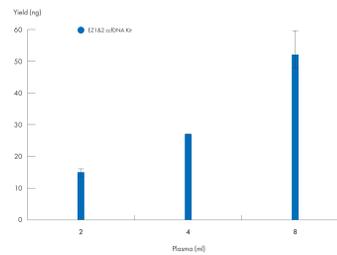
Cancer researchers who field-tested the EZ2 cfDNA isolation system told us that they are facing varying sample amount and therefore need to process different starting volumes. So we adjusted the protocol to easily allow that. Different starting volumes of plasma are processed with the standardized protocols after addition of appropriate volumes of PBS.



**Flexible starting volumes.** Starting volumes were adjusted with PBS to allow processing with the standard protocols. Yields were determined using the Quantiflex Pro RGQ Kit (91 bp human fragment).

### Correlation of yield and sample volume is linear

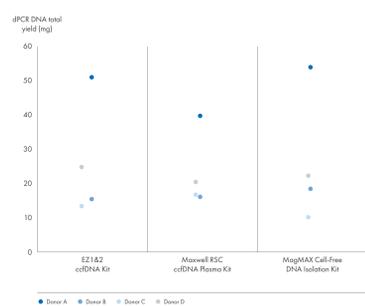
When processing larger volumes, it needs to be ensured that cfDNA is recovered as effectively as from lower volumes. To show this scalability, we analyzed the correlation between sample volume and yield for different starting volumes of plasma.



**Linear DNA yields.** High yields of DNA are isolated from 2, 4 or 8 ml plasma. Yields were determined using the Quantiflex Pro RGQ Kit (91 bp human fragment).

### Yields and dPCR performance

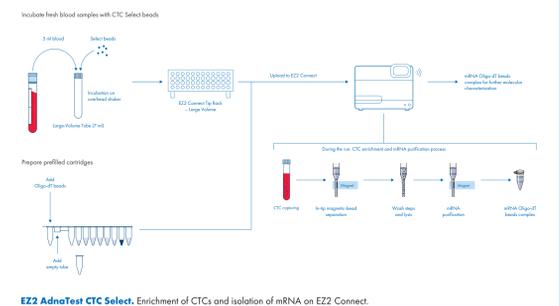
Digital PCR is a technology that is accelerating cancer research with highly sensitive and accurate quantification of biomarkers. To see the relative performance of the EZ1&2 cfDNA Kit on the EZ2 Connect with alternative systems, we compared total yields of wild-type BRAF after amplification by dPCR. The more convenient and fast workflow of the EZ2 system provides cfDNA that is comparably amplifiable in dPCR.



**Comparable yields in dPCR.** Comparison of cfDNA isolation with the EZ1&2 cfDNA Kit, the Maxwell RSC cfDNA Plasma Kit and the MagMAX Cell-Free DNA Isolation Kit show similar yields in dPCR. cfDNA from four different donors was isolated and wild-type BRAF amplified by dPCR using the QIAcuity.

### CTC enrichment and mRNA preparation on the EZ2 Connect

Liquid biopsies are also used to analyze circulating tumor cells (CTCs) to provide important information for cancer research. Therefore, we automated the AdnaTest enrichment of CTCs from whole blood on the EZ2 Connect platform with integrated mRNA isolation for molecular characterization of CTCs. Here, our development aimed for an easy and standardized procedure for CTC enrichment that allows the implementation of CTC research in every lab.



**EZ2 AdnaTest CTC Select.** Enrichment of CTCs and isolation of mRNA on EZ2 Connect.

### Conclusion

With the new automated workflow, the EZ2 Connect system processes liquid biopsies with:

- Faster processing times
- Minimal manual handling
- Flexible starting volumes and linear yields
- Reliable compatibility with dPCR analysis

These data show that the EZ2 Connect workflow simplifies the isolation of cfDNA and analysis of CTCs without compromising yield and quality.

Now you can automate your cfDNA isolation from up to 8 ml of plasma/serum or enrich CTCs from whole blood. The protocols use prefilled cartridges to standardize and reduce human error. Magnetic bead-based, automated nucleic acid isolation from liquid biopsies is easy, fast and flexible.

Data generated by QIAGEN R&D. The methods presented here are intended for molecular biology applications. These methods are not intended for the diagnosis, prevention, or treatment of a disease. For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit instructions for use or user operator manual. QIAGEN kit instructions for use and user manuals are available at [www.qiagen.com](http://www.qiagen.com) or can be requested from QIAGEN Technical Services (or your local distributor).

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