February 2022

EZ2® AdnaTest CTC Select Handbook

For automated enrichment of circulating tumor cells and mRNA isolation using EZ2 Connect

Sample to Insight

QIAGEN

Contents

Kit Contents

EZ2 AdnaTest CTC Select	
Catalog no.	**395692**
Number of tests	**12**
Large Volume Tubes (7 ml)	24
Collection Tubes (1.5 ml)	50
Screw cap collection tubes (1.5 ml)	50
Reagent Cartridge, CTC†	12
CTC-Select Beads† (1.2 ml)	1
Oligo(dT)$_{25}$ Beads (355 µ) †	1
RNase-free Water (1.9ml)	1
Q-Card*	1
Quick-Start Protocol	1

* The information encoded in the bar code on the Q-Card is needed for reagent data tracking using the EZ2 Connect instruments.

† Contains sodium azide as a preservative

The AdnaTest CTC-Select reagents are sufficient to process 12 blood samples.

Shipping and Storage

The EZ2 AdnaTest CTC-Select system is shipped at 2–8°C. The EZ2 reagent cartridges, the CTC-Select beads, and the Oligo(dT)$_{25}$ Beads should be stored at 2–8°C. Under these conditions, the components are stable, without showing any reduction in performance and quality, until the expiry date on the kit label.

Intended Use

The EZ2 AdnaTest CTC Select Kit is for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

The EZ2 AdnaTest CTC Select Kit is intended to be used with the EZ2 Connect instrument from QIAGEN®.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN kit and kit component.

If liquid containing potentially infectious agents is spilt on the EZ2 Connect instrument, please refer to the instrument user manual for decontamination instructions.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of AdnaTest CTC Select is tested against pre-determined specifications to ensure consistent product quality.

Introduction

The EZ2 AdnaTest CTC Select is used for CTC enrichment from human whole blood and subsequent mRNA isolation on the EZ2 Connect instrument. Successful CTC capture is based on a highly specific immunomagnetic cell-selection system using an optimized antibody combination. After the incubation of the blood sample with the AdnaTest Select Beads, the EZ2 Connect instrument performs most steps of the sample preparation procedure. The EZ2 instrument allows to process up to 24 samples in a single run in 35 min.

Principle and Procedure

AdnaTest CTC Select enables the immunomagnetic enrichment of tumor cells via epithelial and tumor-associated antigens. Antibodies are conjugated to magnetic beads for capturing of tumor cells in whole blood. Captured cells are enriched by a magnetic particle concentration on the EZ2 Instrument. Enriched CTCs are lysed and mRNA is purified (Figure 1).

Figure 1. CTC enrichment and mRNA isolation.

Whole blood samples are thoroughly mixed and incubated with magnetic particles to allow adsorption of CTC to the surface. In-tip magnetic bead separation is used to enrich CTCs followed by wash steps using AdnaWash Buffer to reduce leucocyte load. Subsequently, CTCs are lysed and mRNA is isolated using magnetic Oligo(dT)$_{25}$ Beads.

Oligo(dT)$_{25}$ Beads are used for the isolation of mRNA from the lysate of enriched tumor cells. Reverse transcription results in cDNA, which can be used as a template for tumor cell detection and characterization by multiplex PCR. The cancer-specific AdnaTest Add-Ons (see Ordering Information, page 20) allow amplification of cancer-specific/tumor-associated genes and some control genes. The AdnaTest system is an open platform, and therefore, the CTC-derived cDNA can also be used to analyze and identify molecular pathways in cancer research to identify new, relevant biomarkers.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Equipment

- EZ2 Connect (cat. no. 9003210)
- EZ2 Connect Tip Rack - Large Volume (cat. no. 9027011)
- Tube rotator for 15 ml and 1.5 ml tubes (e.g., ELMI Ltd., Intelli-Mixer RM-2L)
- Magnetic particle concentrator, AdnaMag-S (cat. no. 399911)
- Thermal block or water bath (50°C)
- Thermal cycler with a heated lid and a heating rate of 2°C/s.

Material

- EZ1® Filter tips and tip holders (cat. no. 994900)
- Pipettes and RNase-free pipette tips with aerosol barrier, suitable for pipetting volumes from 1 µl to 5 ml.
- Sterile, RNase-free, thin-wall 0.2 ml PCR tubes

Reagents

- Phosphate buffered saline (PBS), pH 7.0–7.3 (e.g., Gibco™ cat. no. 14190169, DPBS)
- Sensiscript® RT Kit (cat. no. 205211, 50 reactions)

  Note: When using the Sensiscript RT Kit with AdnaTest CTC Select, the volume is sufficient for only 25 samples because twice the volume is required for each reaction.
- Recombinant RNasin®, RNase-inhibitor, 2500 U (Promega cat. no. N2511)
- Crushed ice

Important Notes

Sample Preparation

- After receiving the kit, check the kit components for damage. If any kit components are damaged, contact QIAGEN Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (page 4). Do not use damaged kit components, since their use may lead to poor kit performance.
- All steps of the purification procedure should be performed at room temperature (15–25°C).
- Blood samples must be taken before the application of therapeutic substances. Do not use the EZ2 AdnaTest CTC Select earlier than 7 days after the last therapeutic intervention.
- Blood must be stored at 2–8°C immediately.
- Blood collection: If sample transportation is less than 4 hours, EDTA as anticoagulant (e.g., S Monovette® K3 EDTA, Sarstedt cat. no. 01.1605.001) can be used; draw at least 7.5 ml of whole blood.
- If sample transportation is longer than 4 hours, use BD Vacutainer ACD-A Tubes (cat. no. 366645) to draw at least 8.5 ml of whole blood. ACD-A blood can be used within 30 hours from collection.
- The blood sample must not be hemolyzed.

Handling

- AdnaTest CTC-Select Beads contain sodium azide as preservative. Sodium azide is cytotoxic and must, therefore, be removed before using the beads. (See "Protocol: EZ2 AdnaTest CTC Select Kit", page 14).
- All components and additional reagents provided by other suppliers must be stored according to their instructions. Safety advice of the corresponding manufacturers applies.

- Wear protective gloves to avoid contamination with DNA, RNA, and RNases.
- The test must be performed in the sequence described and must comply with all specifications stated with respect to incubation times and incubation temperatures.
- Discard samples if the AdnaTest CTC-Select Beads agglutinate during cell enrichment.
- If possible, perform sample processing, including reverse transcription and subsequent analysis of amplified PCR products, in different rooms, to avoid cross-contamination.
- The use of products from suppliers other than those suggested may adversely affect the results.
- The safety and hygiene regulations of the laboratory must be respected (e.g., wear lab coats, protective goggles, and gloves).

Working with EZ2 Connect instruments

EZ2 Connect reagent cartridges

Reagents for the enrichment of CTCs and their mRNA from a single sample are contained in a single reagent cartridge (Figure 2). Each well of the cartridge contains reagents in a particular order. Position 12 of the cartridge is empty and needs to be filled with Oligo(dT)$_{25}$ Beads, provided with the kit. In position 11 of the cartridge, an empty screw cap tube (provided) needs to be inserted by the user. Details on preparation of these positions are displayed during the run setup on the LED display of the EZ2 Connect.

Figure 2. Ease of worktable setup using reagent cartridges. (A) A sealed, prefilled reagent cartridge. Fill levels vary, depending on the type of reagent cartridge. (B) Loading reagent cartridges into the cartridge rack. The cartridge rack itself is labeled with an arrow to indicate the direction in which reagent cartridges must be loaded.

EZ2 Connect Tip Rack - Large Volume

The EZ2 AdnaTest CTC Select requires the use of 5 ml of blood, which will be processed using the Large Volume Tubes that are provided with the kit. These Large Volume Tubes require the use of the EZ2 Connect Tip Rack - Large Volume (7 ml), which needs to be purchased separately (see “Ordering Information”, page 20). Details on how to equip the tip racks are displayed during the run setup on the LED display of the EZ2 Connect.

Figure 3. EZ2 Connect Tip Rack - Large Volume.

Worktable

The worktable of EZ2 Connect instruments is where the user-equipped cartridge and tip racks are placed (Figure 4). The display also shows protocol status during the automated procedure.

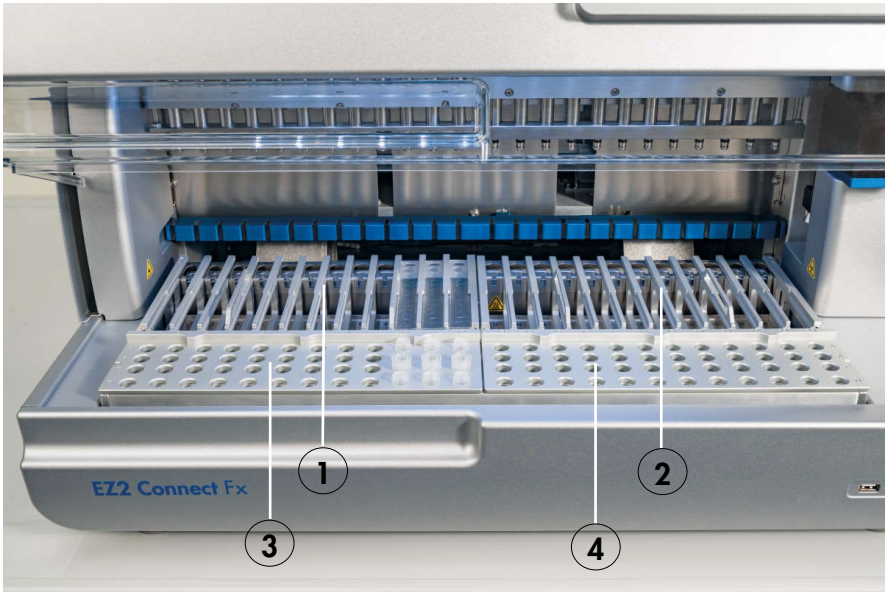

Figure 4. EZ2 Connect Fx Worktable.

1. EZ2 Connect Cartridge Rack – left
2. EZ2 Connect Cartridge Rack – right
3. EZ2 Connect Tip Rack - Large Volume – left
4. EZ2 Connect Tip Rack - Large Volume – right

Operation of the EZ2 Connect

The EZ2 Connect provides various features to support the sample preparation workflow. These include functions for remote access via QIAsphere®, data input via barcode reading, data storage and transfer, report generation, and guided instrument maintenance. For more information about these features, please refer to the *EZ2 Connect and EZ2 Connect Fx User Manual*.

Protocol: EZ2 AdnaTest CTC Select Kit

Important points before starting

- Before beginning the procedure, read "Important Notes" (page 9).
- It is necessary to remove sodium azide by washing the CTC-Select Beads prior to use, as described below in "Preparation of the CTC-Select Beads".
- Make sure you have filter tips and tip holders available; they are not part of the kit and must be purchased separately.

Preparation of the CTC-Select Beads

1. Resuspend the CTC-Select Beads thoroughly by pipetting.

   Important: Do not vortex.

2. Calculate the volume of CTC-Select Beads required for all samples to be processed (100 µl per sample), and transfer the calculated volume into a 1.5 ml reaction tube (not provided).

   If more than 10 samples are processed, use additional 1.5 ml reaction tubes. Do not use more than 1000 µl beads per tube.

3. Place the tube into the AdnaMagS rack.
4. After 1 min, remove the supernatant with a pipette.

   Important: Do not touch the beads when removing the supernatant.

5. Wash steps:

   5a. Remove the magnet slider from the AdnaMagS rack.
   5b. Add 1 ml PBS and resuspend the beads by repeated pipetting.
   5c. Place the magnet slider into the AdnaMag-S rack.
   5d. After 1 min, remove the supernatant completely with a pipette.
   5e. Repeat steps 5a–5d twice (3 washes in total).

Remove the tube from the AdnaMag-S rack, and resuspend the beads in PBS to the original volume (100 µl per sample).

6. When using standard EDTA tubes, transfer 5 ml whole blood into a 7 ml Large Volume Tube (provided). Alternatively, ACD-A blood can be used; transfer 5 ml ACD-A blood into a 7 ml Large Volume Tube and add 94 µl EDTA (0.5 M, pH 8.0), e.g., UltraPure™ 0.5 M EDTA, pH 8.0 (Invitrogen™ cat. no. 15575020).
7. Resuspend the CTC-Select Beads thoroughly (prepared in step 5) by pipetting, and add 100 µl to each blood sample.
8. Incubate tubes for 30 min at room temperature on a device that allows slow rotation (approximately 5 rpm).
9. Turn on the EZ2 Connect instrument.
10. Tap "AdnaTest CTC select" in the RNA Applications panel and follow the instructions on the display.
11. Select sample positions on the work deck and press **Next**.
12. Enter sample IDs or press **Generate missing sample IDs**. Then press **Next**.
13. Load the EZ2 AdnaTest CTC Select reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 11 and allow to equilibrate to room temperature.
14. Remove the cap from an empty 1.5 ml screw cap tube (provided) and place in position 11 of the cartridge (see Figure 1).
15. Thoroughly resuspend the Oligo(dT)$_{25}$ Beads and pipet 20 µl into the bottom of position 12 of the reagent cartridge (see Figure 1).
16. Open instrument hood. Load the cartridge racks and place it into the EZ2 instrument.
17. Prepare the EZ2 Connect Tip Racks - Large Volume as follows:
    - Position A: Tip holder with Filter Tip
    - Position B: Sample in Large Volume Tube from step 6
    - Position C: Empty Large Volume Tube (7 ml, provided)
    - Position D: Tip holder with Filter Tip

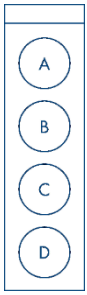

Figure 5. EZ2 Connect Tip Rack.

18. When blood rotation is complete, remove the cap and load the tube in position B of the Tip Rack. Place the EZ2 Connect Tip Rack into the EZ2 Connect instrument. Start the run according to the instructions on the instrument display.
19. The display will show "Protocol finished" when the run is completed. Select **Finish**.
20. After completion of the protocol on the EZ2 instrument, proceed without delay. Remove the 1.5 ml screw cap tube from position 11 and transfer the entire volume (500 µl) to a fresh 1.5 ml reaction tube (provided).
21. Place it on a AdnaMag-S rack. Wait for 1 min and carefully remove the supernatant.
22. Remove the magnet from the AdnaMag-S rack and resuspend beads in 29.5 µl RNase-free H_2O.

    Note: If using the AdnaTest Add-On Prostate or AR-V7, please resuspend beads in 14.75 µl RNase-free H_2O.
23. Incubate the beads at 50°C for 5 min. Then, place on ice immediately for 2 min.
24. Proceed with reverse transcription within 5 min; See Table 1 and Table 2.

Reverse transcription using the Sensiscript RT Kit

1. Prepare the RT Master Mix on ice. The RT Master Mix is prepared, as shown in Table 1 according to the number of samples.

   The volume of RT Master Mix should be 10% greater than calculated for the total number of reverse-transcription reactions. A negative control reaction without addition of mRNA must always be prepared (RT control).

Table 1. Reverse transcription reaction setup

Component		AdnaTest Add-On Prostate or AR-V7
	Volume	Volume
RT Master Mix		
10x Buffer RT	4.0 µl	2.0 µl
dNTP Mix (5 mM each dNTP)	4.0 µl	2.0 µl
RNase inhibitor, 40 U/µl (Promega)	0.5 µl	0.25 µl
Sensiscript Reverse Transcriptase	2.0 µl	1.0 µl
Template RNA* mRNA/bead complex or RNase-free water	**29.5 µl**	**14.75 µl**
Total volume	**40.0 µl**	**20.0 µl**

* As RT control, add 29.5 µl, in case for prostate and AR-V7 14.75 µl, of RNase-free water instead of mRNA/bead complex. The volume of the mRNA/bead complex may vary slightly. Always use the total volume of this in the reverse transcription reaction.

2. Vortex the RT Master Mix. Centrifuge briefly, and pipet 10.5 µl, in case of prostate and AR-V7 5.25 µl, for each reaction into 0.2 ml PCR tubes.
3. Resuspend the mRNA/bead complexes (step 23, page 16) carefully with a pipette. Transfer the total volume into the 0.2 ml PCR tube containing the RT Master Mix. Mix thoroughly by repeated pipetting.
4. cDNA is synthesized in a thermal cycler under the following conditions (Table 2).

Table 2. Reverse transcription program

Step	Time	Temperature
Reverse transcription	60 min	37°C
Denaturation	5 min	93°C
Cooling	∞	4°C

Place reaction tubes with the cDNA/bead complex on ice for immediate processing, or store at –30 to –15°C for a maximum of 4 weeks.

5. Continue with the AdnaTest Add-On of choice (see “Ordering Information” for options) tumor marker profiling using your in-house methods of choice or using commercial available panels or primer set (GeneGlobe).

Troubleshooting Guide

See the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

		Comments and suggestions
General handling		
a)	Error message in instrument display	Refer to the user manual supplied with your EZ2 Connect instrument.
Low mRNA yield/Low performance		
a)	Magnetic particles not completely resuspended	Ensure that you mix by pipetting repeatedly up and down (do not vortex) the tube containing the magnetic beads several times to resuspend the magnetic particles.
b)	Varying pipetting volumes	To ensure pipetting accuracy, it is important that buffer volumes in the reagent cartridges are correct and that the filter tips fit optimally to the tip adapter. Ensure that samples are thoroughly mixed and that reagent cartridges have not passed their expiry date. Perform regular maintenance as described in the instrument user manual. Check the fit of the filter tips regularly as described in the user manual.

Ordering Information

Product	Contents	Cat. no.
EZ2 AdnaTest CTC Select	For isolation of CTCs and the subsequent extraction of mRNA from human whole blood for 12 preparations	395692
EZ2 Connect	Benchtop instrument for automated isolation of nucleic acids from up to 24 samples in parallel, using sealed prefilled cartridges; includes 1-year warranty on parts and labor.	9003210
EZ2 Connect Tip Rack - Large Volume	Rack for the EZ2 Connect	9027011
Filter-Tips and Holders, EZ1 (50)	50 Disposable Filter-Tips, 50 Disposable Tip Holders; additional tips and holders for use with EZ1, EZ1&2™, and EZ2 Kits	994900
AdnaMag-S	Magnetic rack for 8 x 1.5 ml tubes	399911
AdnaTest Add-on BreastCancerDetect	PrimerMix for 12 PCR reactions	396212
AdnaTest Add-on ColonCancerDetect	PrimerMix for 12 PCR reactions	396222
AdnaTest Add-on ProstateCancerDetect	PrimerMix for 12 PCR reactions	396232
AdnaTest Add-on OvarianCancerDetect	PrimerMix for 12 PCR reactions	396242

Product	Contents	Cat. no.
Related products		
AdnaTube	12 sample tubes containing EDTA. Use only with anticoagulated blood collected in ACDA blood collection tubes from BD (Becton Dickinson, 8.5 ml)	399932
Sensiscript RT Kit (50)	For 50 reverse-transcription reactions*: Sensiscript Reverse Transcriptase, 150 µl 10x Buffer RT, 100 µl dNTP Mix (contains 5 mM of each dNTP), 1.1 ml RNase-free water	205211

* The Sensiscript RT Kit (50) is sufficient for only 25 samples using AdnaTest CTC Select because twice the volume is required for each reaction.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Date	Changes
02/2022	Initial revision

Limited License Agreement for AdnaTest CTC Select

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