



AdnaTest BreastCancerSelect

**Enrichment of tumor cells
from blood of breast cancer patients
for gene expression analysis**

For in vitro diagnostic use

Manual



Article no. 81005 / 81021 / 81024

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Order Information

AdnaTest BreastCancerSelect and *AdnaTest BreastCancerDetect* are developed and manufactured by AdnaGen AG and distributed, sold and marketed by Innogenetics N.V..

| | Specifications | Order no. |
|------------------------------------|----------------|-----------|
| <i>AdnaTest BreastCancerSelect</i> | 12 Selections | 81005 |
| | 24 Selections | 81021 |
| | 36 Selections | 81024 |
| <i>AdnaTest BreastCancerDetect</i> | 12 Detections | 81004 |
| | 24 Detections | 81019 |
| | 36 Detections | 81022 |
| <i>AdnaCollect</i> | 12 Systems | 81003 |



Purpose

AdnaTest BreastCancerSelect is for in vitro diagnostic use only and was developed for the enrichment of circulating tumor cells from peripheral blood of breast cancer patients.

AdnaTest BreastCancerDetect is recommended for the subsequent analysis of the breast cancer associated gene expression.

Further information can be found on the website www.adnagen.com.

Abbreviations and Symbols

| | |
|---|---|
| bp | base pairs |
| cDNA | complementary deoxyribonucleic acid |
| DNA | deoxyribonucleic acid |
| MPC (-S, -L) | magnetic particle concentrator (-small, -large) |
| mRNA | messenger ribonucleic acid |
| PCR | polymerase chain reaction |
| RNase | ribonuclease |
| rpm | revolutions per minute |
| RT | reverse transcription |
|  | expiry date |
|  | storage temperature |

Patents and Registered Trademarks

Dynabeads is a registered trademark of Dynal Biotech ASA, Oslo, Norway.

Product Description

AdnaTest BreastCancerSelect enables the immunomagnetic enrichment of tumor cells via epithelial and tumor associated antigens. Antibodies against epithelial and tumor associated antigens are conjugated to magnetic beads (Dynabeads) for the labeling of tumor cells in peripheral blood. The labeled cells are extracted by a magnetic particle concentrator (MPC) and are subsequently lysed (Figure 1).

The cell lysate is used for further analysis (it is recommended to continue with *AdnaTest BreastCancerDetect*).

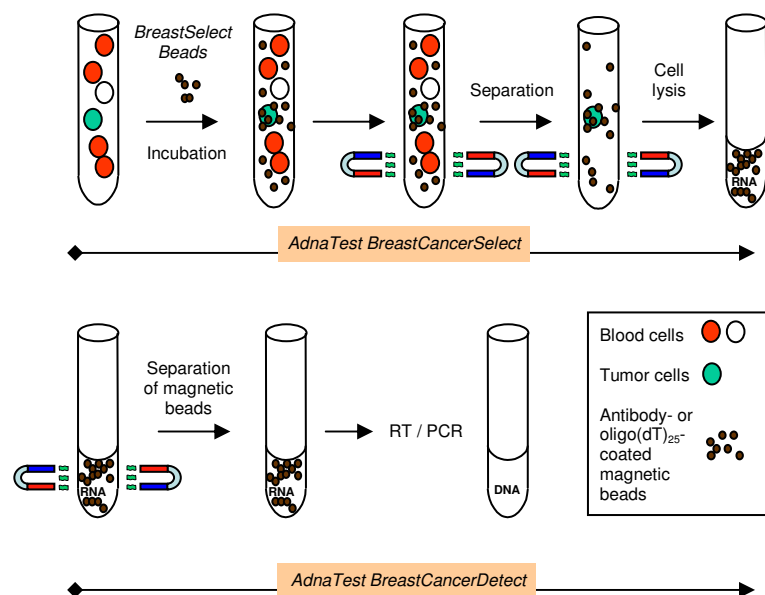


Figure 1: Schematic overview of the sample preparation

Kit Components

AdnaTest BreastCancerSelect includes the following components (number of tubes):

Table 1: Kit components

| Component | Symbol | 81005 (12 tests) | 81021 (24 tests) | 81024 (36 tests) |
|-----------------------------|--------|---------------------|---------------------|---------------------|
| <i>BreastSelect Beads</i> | 1 | 1 | 2 | 3 |
| <i>Lysis/Binding Buffer</i> | 2 | 1 | 1 | 1 |

Additional Materials Needed

Equipment:

- Tube rotator for 15 ml and 1.5 ml tubes
- Magnetic Particle Concentrators MPC-L (DynaL MPC-L, Invitrogen, cat. no. 120-21D) and MPC-S (DynaL MPC-S, Invitrogen, cat. no. 120-20D)

Material:

- Sterile, RNase-free glass or plastic 10 ml pipets and pipettor
- Sterile, RNase-free 1.5 ml reaction tubes
- 15 ml centrifuge tubes (use sterile, RNase-free polypropylene tubes)
- Pipets (100 - 1,000 μ l), RNase-free pipet tips with aerosol barrier
- Protective gloves, safety goggles

Reagents:

- Phosphate buffered saline (PBS), pH 7.2 (Invitrogen, cat no. 14190-094, D-PBS)

Storage

AdnaTest BreastCancerSelect has to be stored at 4 °C and must not be used beyond the expiry date.

Application Information

The test must be performed by personnel skilled in molecular biological techniques.

Sample Preparation

- Blood samples must be taken before the application of therapeutic substances. Do not use the *AdnaTest* earlier than 5 days after the last therapeutic intervention!
- Blood withdrawal: Use ***AdnaCollect*** blood collection tubes (prod. no. T-1-600, AdnaGen) or tubes containing **EDTA** as anticoagulant for blood withdrawal ('S-Monovette® Kalium EDTA', Sarstedt; 'BD Vacutainer® K₃EDTA', Becton Dickinson). Draw at least 5 ml blood.
- Blood has to be placed on ice immediately and stored in the cold (4 °C).
- **Samples must be processed immediately but not later than 4 hours after blood withdrawal when using standard EDTA tubes or within 24 hours when using *AdnaCollect*.**
- The blood sample must not be haemolysed.

Handling

- *BreastSelect Beads* **1** contain sodium azide as preservative. Sodium azide is cytotoxic and must, therefore, be removed before using the beads.
- All components and additional reagents provided by other suppliers have to be stored according to the instructions. Safety advices of the respective manufacturers are valid.
- Wear protective gloves to avoid contamination with DNA, RNA and RNases.
- Aliquote the *BreastSelect Beads* to avoid contamination.
- Processing has to be performed in the denoted sequence and has to comply with all specifications stated with respect to incubation times and incubation temperatures.
- Discard samples if the selection beads agglutinate during cell enrichment.
- Perform sample processing and subsequent analysis of amplified PCR products in different rooms to avoid cross-contamination.
- The safety and hygiene regulations of the laboratory must be respected (e. g. wear lab coats, protective goggles, gloves).

Protocol

A Preparation of the BreastSelect Beads

It is necessary to remove the sodium azide by washing the *BreastSelect Beads* prior use:

1. Resuspend the *BreastSelect Beads* **1** thoroughly by pipetting; do not vortex!
2. Calculate the volume of *BreastSelect Beads* **1** required for all samples to be processed (100 µl per sample) and transfer the calculated volume into a 1.5 ml reaction tube.
If more than 10 samples are processed use additional 1.5 ml reaction tubes.
3. Place the tube into a MPC-S.
4. After 1 min remove the supernatant with a pipet.

Important for each procedure:

Do not touch the beads when you remove the supernatants!

5. Washing
 - a. Remove the magnet from the MPC-S.
 - b. Add 1 ml PBS (pH 7.2) and resuspend the beads by repeated pipetting.
 - c. Place the magnet into the MPC-S.
 - d. After 1 min remove the supernatant completely.
Repeat twice (Three washings in total).
6. Remove the tube from the magnet and resuspend the beads in PBS (pH 7.2) to the original volume and store on ice.

B Selection of Tumor Cells

1. Pipet 5 ml of a blood sample into a 15 ml reaction tube.
(Use approved blood collection tubes only, see page 7)
2. Resuspend the *BreastSelect Beads* by pipetting (prepared in step A6) and add 100 µl of these beads to each blood sample.
3. Rotate the tubes slowly (approx. 5 rpm) for 15 – 30 min at room temperature on a device allowing both tilting and rotation.
4. Place the tubes into the MPC-L without magnet. Swing the MPC-L downwards to release cap-captured blood drops to the tube.
5. Insert the magnet and incubate the tubes in the MPC-L for 3 min at room temperature.
6. In the meantime equilibrate *Lysis/Binding Buffer* **2** to room temperature.

Note: Check that the *Lysis/Binding Buffer* contains no precipitate. If any precipitate is observed, equilibrate the buffer to room temperature and shake until it is completely dissolved.

7. Remove the blood supernatant completely with a 10 ml pipet without touching the beads.
8. Washing
 - a. Remove the magnet from the MPC-L.
 - b. Add 5 ml PBS (pH 7.2), close the tubes and rock back and forth slowly and gently for 5 times on each side of the MPC-L to resuspend the magnetic bead/cell complexes.

- c. Swing the MPC-L with the tubes downwards twice to release cap-captured drops.
- d. Place the magnet into the MPC-L and incubate for 1 min at room temperature.
- e. Remove the supernatant completely.

Repeat twice (three washings in total).

9. Remove the magnet from the MPC-L.
10. Resuspend the magnetic bead/cell complexes in 1 ml PBS (pH 7.2) and transfer each sample into a 1.5 ml reaction tube.
11. Place the reaction tubes into the MPC-S with an inserted magnet.
Note: In the MPC-S the magnet can be inserted in two positions. Always use the front position to make sure that the magnet is close to the reaction tube.
12. After 1 min remove the supernatants **completely** to optimize the following cell lysis!
13. Remove the magnet from the MPC-S.
14. Add 200 µl *Lysis/Binding Buffer* 2 (RT) to each reaction tube.
Resuspend by pipetting at least five times.
15. Place the magnet into the MPC-S and incubate for 1 min.
16. Transfer the supernatants (cell lysates) into new 1.5 ml reaction tubes.
17. Discard the tubes with the beads.
18. Continue with the mRNA-isolation immediately (*AdnaTest BreastCancerDetect*) or store the lysate at -20 °C not longer than 2 weeks.

References

For references, please, refer to our website.

<http://www.adnagen.com>

Short Manual

AdnaTest *BreastCancerSelect*

| | | |
|--------------------------------|--|----------|
| Component | <i>BreastSelect Beads</i> | 1 |
| | <i>Lysis/Binding Buffer</i> | 2 |
| You need for one sample | 5 ml EDTA-blood 1x 15 ml Reaction tube 2x 1.5 ml Reaction tubes 10 ml RNase-free glass or plastic pipets 100 – 1,000 µl RNase-free pipets and tips | |

Protocol

- Resuspend the *BreastSelect Beads* **1** (100 µl per sample) thoroughly and transfer 100 µl for each blood sample into a 1.5 ml reaction tube.
- Wash the *BreastSelect Beads* with 3x 1 ml PBS.
- Resuspend the *BreastSelect Beads* in 100 µl PBS per blood sample.
- Transfer 5 ml EDTA-blood into a 15 ml reaction tube.
- Add 100 µl of the washed *BreastSelect Beads* to each blood sample.
- Incubate for 15 - 30 min at room temperature under tilting and rotation at 5 rpm.
- Place the tube for 3 min in MPC-L to separate the beads. Release any cap-captured droplets by swinging the MPC-L downwards.
- Remove blood supernatant.
- Wash the beads with 3 x 5 ml PBS.

- Resuspend the beads in 1 ml PBS and transfer into a new 1.5 ml reaction tube.
- Separate the beads in the MPC-S and remove the supernatant.
- Resuspend the beads in 200 µl Lysis/Binding Buffer [2] by pipetting at least five times.
- Place the reaction tubes into the MPC-S and transfer the supernatant into a new reaction tube.

Continue immediately with the AdnaTest *BreastCancerDetect* or store at -20 °C for max. 2 weeks.

AdnaTest BreastCancerSelect is distributed by:

INNOGENETICS N.V. Phone: +32 9 329 16 11
 Technologiepark 6
 B-9052 Zwijnaarde, BELGIUM

Technical Support:

INNOGENETICS GmbH Phone: +49 2867 990 731
 Lembeckerstrasse 19
 D-46359 Heiden
 GERMANY

INNOGENETICS Phone: +34 93 270 53 11
 DIAGNOSTICA IBERIA
 S.L. Unipersonal
 C/Tarragona, 161, planta 14
 08014 Barcelona
 SPAIN

INNOGENETICS s.a.r.l. Phone: +33 1 69 07 48 34
 Z.A. Courtaboeuf
 Les Conquérants, Bât. Le Kilimandjaro
 8/10 av. des Tropiques
 F-91940 Les Ulis
 FRANCE

INNOGENETICS Srl Phone: +39 06 911 80 375
 Via del mare 36
 I-00040 Pomezia
 ITALY