



# *AdnaTest* *EMT-1/Stem CellSelect*

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

*For research use only*

## **Manual**

Article no. T-1-533

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## Order Information

On the website [www.adnagen.com](http://www.adnagen.com) the addresses of distributors and information about our products can be found. Our distributors will provide you as well with technical support.

Furthermore, AdnaGen's team will answer you any questions regarding the *AdnaTests* (support@adnagen.com).

*AdnaTest EMT-1/Stem Cell* can be ordered as listed below.



	Specifications	Order no.
<i>AdnaTest EMT-1/Stem Cell</i>	12 tests	T-1-533

## Purpose

*AdnaTest EMT-1/Stem CellSelect* is for research use only and was developed for the enrichment of circulating tumor cells from peripheral blood.

*AdnaTest EMT-1/Stem CellDetect* is required for the subsequent analysis of the EMT-1/Stem Cell associated genes expression.

## Abbreviations and Symbols

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

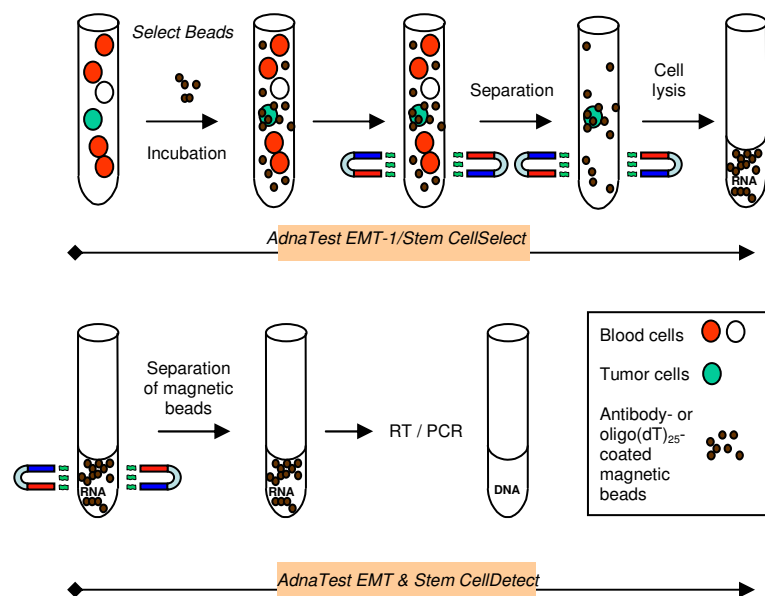
## Patents and Registered Trademarks

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

## Product Description

*AdnaTest EMT-1/Stem CellSelect* 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 (*AdnaMag* and MPC-S) and are subsequently lysed (Figure 1).

The cell lysate is used for further analysis with *AdnaTest EMT-1/Stem CellDetect*.



**Figure 1: Schematic overview of the sample preparation**

## Kit Components

*AdnaTest EMT-1/Stem CellSelect* includes the following components (number of tubes):

**Table 1: Kit components**

Component	Symbol	T-1-533 (12 tests)
Select Beads	1	1
Lysis/Binding Buffer	2	1
AdnaWash	15	2

## Additional Materials Needed

Equipment:

- Tube rotator for 15 ml and 1.5 ml tubes
- Magnetic Particle Concentrators *AdnaMag* (AdnaGen AG, cat. no. T-1-700) and 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 µl), 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 EMT-1/Stem CellSelect* has to be stored at 4 °C. **However, store *AdnaWash* [15] separately at -20 °C.** In order to prevent possible contaminations and repeated temperature changes aliquot the *AdnaWash*. All components 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® K<sub>3</sub>EDTA', 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

- *Select 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 *Select 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 Select Beads

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

1. Resuspend the *Select Beads* [1] thoroughly by pipetting; do not vortex!
2. Calculate the volume of *Select 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 *Select Beads* (prepared in step A 6) by pipetting (prepared in step A 6) 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 *AdnaMag*. Swing the *AdnaMag* downwards to release cap-captured blood drops to the tube.
5. Incubate the tubes in the *AdnaMag* for 3 min at room temperature.
6. In the meantime equilibrate *AdnaWash* [15] and *Lysis/Binding Buffer* [2] to room temperature.

**Note:** Check that the *Lysis/Binding Buffer* and the *AdnaWash* 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 *AdnaMag*.
  - b. Add 5 ml *AdnaWash*, close the tubes and rock back and forth slowly and gently until the beads are resuspended again properly.

- c. Swing the *AdnaMag* with the tubes downwards twice to release cap-captured drops.
- d. Place the magnet into the *AdnaMag* and incubate for 3 min at room temperature.
- e. Remove the supernatant completely.

**Repeat twice (three washings in total).**

9. Remove the magnet from the *AdnaMag*.
10. Resuspend the magnetic bead/cell complexes in 1 ml *AdnaWash* 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 3 min remove the supernatants completely.
13. Remove the magnet from the MPC-S.
14. Resuspend the magnetic bead/cell complexes in 1 ml PBS (pH 7.2)
15. Place the magnet into MPC-S
16. After 1 min remove the supernatants **completely** to optimize the following cell lysis!
17. Remove the magnet from the MPC-S.
18. Add 200 µl *Lysis/Binding Buffer* 2 (RT) to each reaction tube. Resuspend by pipetting at least five times.
19. Place the magnet into the MPC-S and incubate for 1 min.
20. Transfer the **supernatants** (cell lysates) into new 1.5 ml reaction tubes.

21. Discard the tubes with the beads.
22. Continue with the mRNA-isolation immediately (*AdnaTest EMT-1/Stem CellDetect*) 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 EMT-1/Stem CellSelect

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

- Resuspend the beads in 1 ml *AdnaWash*, transfer into a new 1.5 ml reaction tube and incubate for 3 min in the MPC-S
- Remove the supernatant and wash with PBS
- Incubate for 1 min, 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 EMT-1/Stem CellDetect or store at -20 °C for max. 2 weeks.**

## Protocol

- Resuspend the *Select Beads* 1 (100 µl per sample) thoroughly and transfer 100 µl for each blood sample into a 1.5 ml reaction tube.
- Wash the *Select Beads* with 3x 1 ml PBS.
- Resuspend the *Select 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 *Select 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 *AdnaMag* to separate the beads. Release any cap-captured droplets by swinging the *AdnaMag* downwards.
- Remove blood supernatant.
- Wash the beads with 3 x 5 ml *AdnaWash*.

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