

150604 EN

AdnaTest *EMT-2/StemCellSelect*

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

For research use only

Manual

REF T-1-537

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Order Information

On the website www.adnagen.com the addresses of distributors and information about our products can be found. Our distributors will provide you also with technical support.

Furthermore, the QIAGEN Hannover support team will answer you any questions regarding the *AdnaTests* (support@adnagen.com).

Available Add-ons (12 tests):

Article No.	Tumor	Genes
T-1-537-PB	EMT-2 Add-on Breast	GA733-2, Her2, Muc-1
T-1-537-PC	EMT-2 Add-on Colon	GA733-2, CEA, EGFR
T-1-537-PP	EMT-2 Add-on Prostate	PSMA, PSA, EGFR
T-1-537-PO-2	EMT-2 Add-on Ovarian-2	CA125, GA733-2, Muc-1

Purpose






AdnaTest EMT-2/StemCellSelect was developed for the enrichment of circulating tumor cells from peripheral blood and is intended for research use only.

AdnaTest EMT-2/StemCellDetect is required for the subsequent analysis of the EMT-2/StemCell associated genes expression. The specificity of the detection is 90%. Using EMT-2 markers in spiking experiments 20 tumor cells in 5 ml of whole blood are detected at a recovery rate of at least 70%. Using StemCell markers in spiking

experiments 10 tumor cells in 5 ml of whole blood are detected at a recovery rate of at least 70%.

Optionally the analysis of cancer associated genes for the entities Breast, Colon, Prostate and Ovarian is possible (q.v. Order Information, available EMT-2 Add-ons).

Abbreviations and Symbols

<i>AdnaMag-L</i>	Magnetic particle concentrator (-large)
<i>AdnaMag-S</i>	Magnetic particle concentrator (-small)
bp	Base pairs
cDNA	Complementary deoxyribonucleic acid
DNA	Deoxyribonucleic acid
mRNA	Messenger ribonucleic acid
PCR	Polymerase chain reaction
RNase	Ribonuclease
rpm	Revolutions per minute
RT	Reverse transcription
	Expiry date
	Storage temperature
	Catalogue number
	Consider instructions for use
	Manufactured by

Patents and Registered Trademarks

Dynabeads® is a registered trademark of Invitrogen and Life Technologies Corporation.

Product Description

AdnaTest EMT-2/StemCellSelect 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 labeling of tumor cells in peripheral blood. Labeled cells are extracted by a magnetic particle concentrator (*AdnaMag-L* and *AdnaMag-S*) and are subsequently lysed (Figure 1).

The cell lysate is used for further gene expression analysis with *AdnaTest EMT-2/StemCellDetect*.

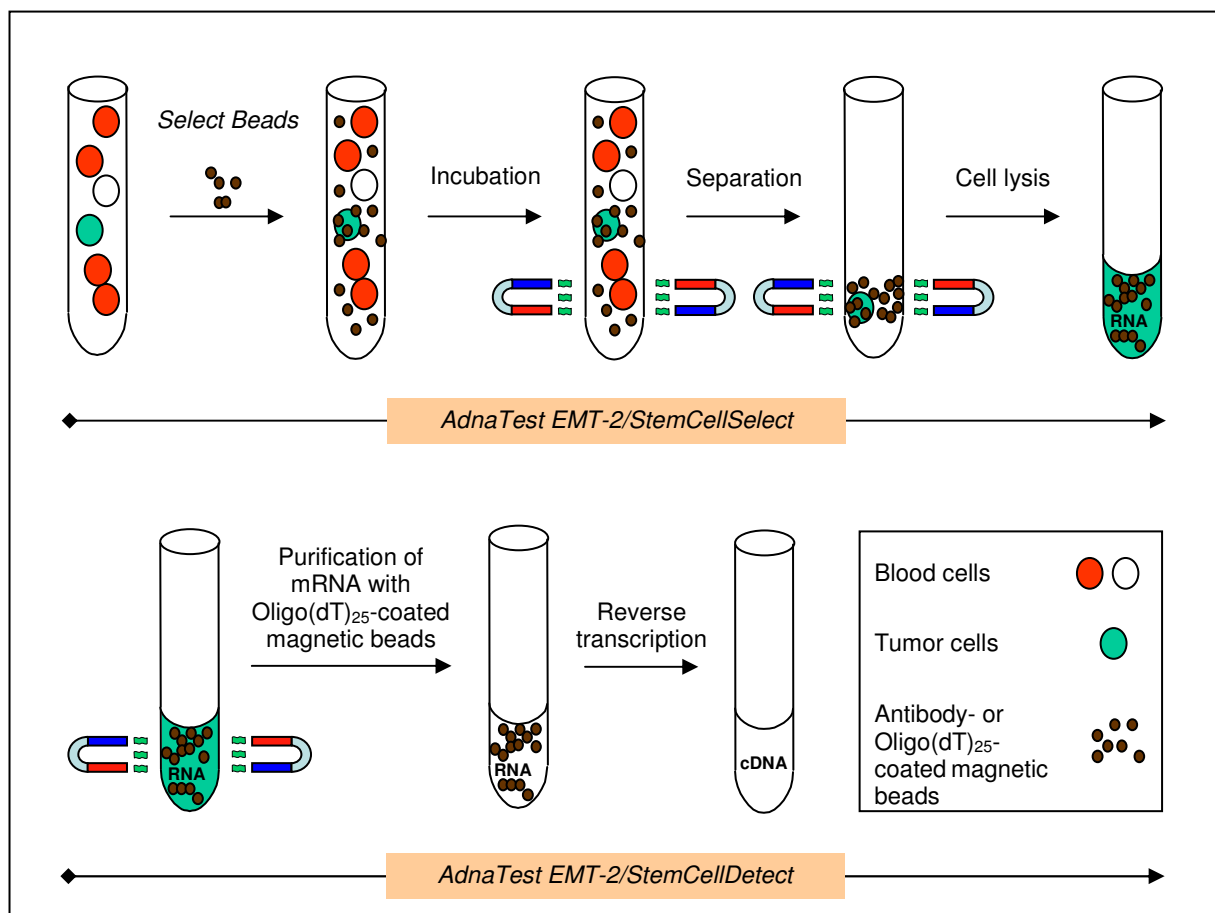


Fig. 1: Schematic overview of the sample preparation

Kit Components

AdnaTest EMT-2/StemCellSelect includes the following components:

Table 1: Kit components

Component	Symbol	T-1-537 (12 tests)
<i>Select Beads</i>	1	1
<i>Lysis/Binding Buffer</i>	2	1
<i>AdnaWash</i>	15	2

Additional Materials Needed

Equipment:

- Tube rotator for 15 ml and 1.5 ml tubes
- Magnetic particle concentrators
 - AdnaMag-L* (QIAGEN Hannover GmbH, cat. no. T-1-700)
 - AdnaMag-S* (QIAGEN Hannover GmbH, cat. no. T-1-800)

Material:

- Sterile, RNase-free 10 ml glass or plastic pipets and pipettor
- Sterile, RNase-free 1.5 ml reaction tubes (e. g. Sarstedt, cat. no. 72.690)
- 15 ml tubes (use sterile, RNase-free polypropylene tubes e. g. Sarstedt, cat. no. 65.554.502)
- Pipets and RNase-free pipet tips with aerosol barrier, suitable for pipetting volumes from 100 µl to 1000 µl
- Protective gloves, safety goggles

Reagents:

- Phosphate buffered saline (PBS), pH 7.0 - 7.3 (e. g. Fisher, cat no. VX14190169, D-PBS)

Storage

AdnaTest EMT-2/StemCellSelect has to be stored at +4 °C. However, in order to prevent possible contaminations and repeated temperature changes, aliquot the *AdnaWash* [15] and keep frozen at -20 °C for long-term storage. 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, QIAGEN Hannover GmbH) or tubes containing EDTA as anticoagulant for blood withdrawal (e. g. 'S Monovette® Kalium EDTA', Sarstedt; 'BD Vacutainer® K3EDTA', Becton Dickinson) to draw at least 5 ml of whole blood.
- Blood has to be stored at 4 °C immediately.
- **Samples should be processed as soon as possible, 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 hemolyzed.

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 their instructions. Safety advices of the respective manufacturers are valid.
- Wear protective gloves to avoid contamination with DNA, RNA and RNases.
- Aliquot the *Select Beads* to avoid contamination.



The test has to be performed in the denoted sequence and has to comply with all specifications stated in respect of incubation times and incubation temperatures.

- Discard samples if the selection beads agglutinate during cell enrichment.
- Perform sample processing incl. reverse transcription and subsequent analysis of amplified PCR products in different rooms, if possible, to avoid cross-contamination.
- **The use of products from other suppliers than suggested may cause inferior results.**
- 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 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 the *AdnaMag-S*.
4. After 1 min remove the supernatant with a pipet.

Important for each procedure:

Do not touch the beads when removing the supernatants!

5. Washing
 - a. Remove the magnet slider from the *AdnaMag-S*.
 - b. Add 1 ml PBS and resuspend the beads by repeated pipetting.
 - c. Place the magnet slider into the *AdnaMag-S*.
 - d. After 1 min remove the supernatant completely with a pipet.**Repeat twice (three washings in total).**
6. Remove the tube from the *AdnaMag-S* and resuspend the beads in PBS to the original volume (100 µl per sample).

B. Selection of Tumor Cells

1. Pipet 5 ml of a blood sample into a 15 ml tube. (Use approved blood collection tubes only, see page 9)
2. Resuspend the *Select Beads* thoroughly (prepared in step A6) by pipetting and add 100 µl of these beads to each blood sample.
3. Rotate tubes slowly (approx. 5 rpm) for 30 min at room temperature on a device allowing both tilting and rotation.
4. Place tubes into the *AdnaMag-L* without magnet slider. Swing the *AdnaMag-L* downwards to release blood drops captured in the cap.
5. Insert magnet slider and incubate the tubes in the *AdnaMag-L* 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* contain no precipitate. If any precipitate is observed, equilibrate the buffer to room temperature and mix until it is completely dissolved.

7. Remove blood supernatant completely with a 10 ml pipet without touching the beads.
8. Washing
 - a. Remove magnet slider from the *AdnaMag-L*.
 - b. Add 5 ml *AdnaWash*, close the tubes and shake the *AdnaMag-L* gently back and forth 5 times to resuspend the magnetic bead/cell complexes
 - c. Swing the *AdnaMag-L* with the tubes downwards twice to release drops captured in the cap.

d. Place magnet slider into the *AdnaMag-L* and incubate for 3 min at room temperature.

e. Remove supernatant completely with a pipet.

Repeat twice (three washings in total).

9. Remove magnet slider from the *AdnaMag-L*.

10. Resuspend magnetic bead/cell complexes in 1 ml *AdnaWash* and transfer each sample into a 1.5 ml reaction tube.

11. Place reaction tubes into the *AdnaMag-S* with an inserted magnet slider.

Note: The magnet slider of the *AdnaMag-S* can be inserted in two positions. Always insert the slider with forward-facing white plastic film to make sure that the magnets are close to the reaction tubes.

12. After 3 min remove supernatants with a pipet.

13. Remove magnet slider from the *AdnaMag-S*.

14. Resuspend the magnetic bead/cell complexes in 1 ml PBS.

15. Place the magnet into *AdnaMag-S*.

16. After 1 min remove the supernatants **completely** with a pipet to optimize the following cell lysis!

17. Remove the magnet from the *AdnaMag-S*.

18. Add 200 µl *Lysis/Binding Buffer* [2] (equilibrated to room temperature) to each reaction tube. Resuspend by pipetting at least five times.

19. Insert magnet slider into the *AdnaMag-S* and incubate for 1 min.

20. Transfer supernatants (cell lysates) into new 1.5 ml reaction tubes.

21. Discard the tubes with the beads.

22. Continue with mRNA-isolation immediately (*AdnaTest EMT-2/ StemCellDetect*) or store the cell lysates at -20 °C no longer than 2 weeks.

References

For references please refer to our website

<http://www.adnagen.com>

Short Manual

AdnaTest EMT-2/StemCellSelect

Components	<i>Select Beads</i>	1
	<i>Lysis/Binding Buffer</i>	2
	<i>AdnaWash</i>	15
For each sample you need	<ul style="list-style-type: none">• 5 ml whole blood (see page 9 for details)• 1x 15 ml tube• 2x 1.5 ml reaction tube• 10 ml glass or plastic pipets (RNase-free) and pipettor• 100 - 1000 µl pipets and tips (RNase free)	

Protocol

- Resuspend *Select Beads* 1 thoroughly and transfer 100 µl for each blood sample into a 1.5 ml reaction tube.
- Wash *Select Beads* with 3x 1 ml PBS.
- Resuspend *Select Beads* in 100 µl PBS per blood sample.

- Transfer 5 ml whole blood into a 15 ml tube.
- Add 100 µl of washed *Select Beads* to each blood sample.
- Incubate for 30 min at room temperature under tilting and rotation at approx. 5 rpm.
- Place tube for 3 min in *AdnaMag-L* to separate the beads. Release any blood drops captured in the cap by swinging the *AdnaMag-L* downwards.
- Remove blood supernatant.
- Wash beads with 3 x 5 ml *AdnaWash*.
- Resuspend beads in 1 ml *AdnaWash* and transfer into a new 1.5 ml reaction tube.
- Separate beads for 3 min in the *AdnaMag-S* and remove supernatant.
- Wash beads with 1 ml PBS.
- Separate beads for 1 min in the *AdnaMag-S* and remove supernatant.
- Resuspend beads in 200 µl *Lysis/Binding Buffer* [2] by pipetting at least five times.
- Place reaction tubes into the *AdnaMag-S* for 1 min and transfer supernatant into a new reaction tube.

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



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