Curious about what CTCs could tell you?

Sample to Insight

Gain new insights by discovering the

Why care about circulating tumor cells?

The importance of circulating tumor cells (CTCs) is a heavily discussed topic in scientific research. But why should you care about CTCs in the first place? CTCs play a crucial role in tumor dissemination. They carry a wealth of valuable information about disease progression.

More specifically, CTCs can tell you about:

- Biology of tumor cells
- Molecular profile of tumor cells
- Stem cell renewal
- Mechanisms of cancer progression
- New, relevant biomarkers
- Relevant molecular pathways in cancer

CTCs provide a good indication of disease progression because they undergo phenotypic changes that are collectively known as the epithelial-to-mesenchymal transition (EMT). These changes allow them to travel to sites of metastasis formation – making detection of metastasis easier for you. Since CTCs are mostly resistant to cytotoxic agents, they are not destroyed or affected by conventional tumor therapy. It's not surprising that the presence of CTCs in the blood correlates with poor outcomes and lower survival rates in a number of studies. Your study of molecular mechanisms regulating CTCs and EMT in solid tumors can provide new insights into stem cell renewal and the mechanisms of cancer progression.

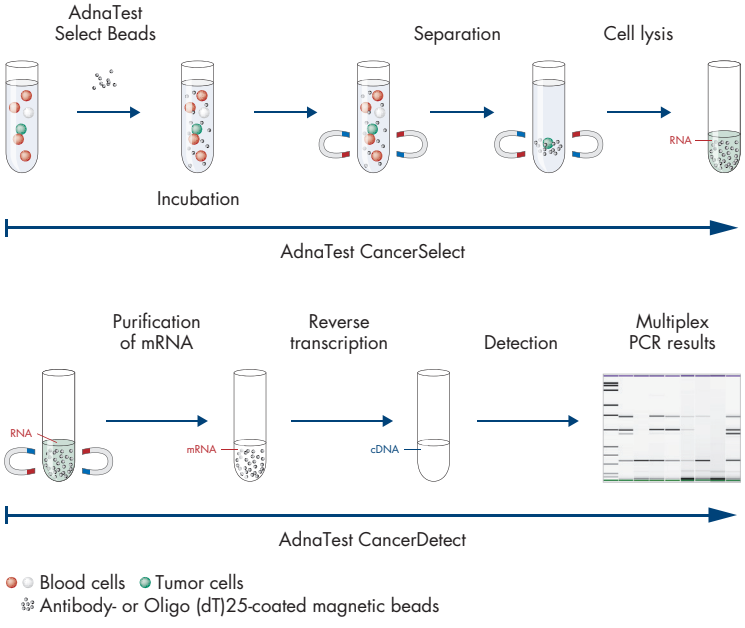
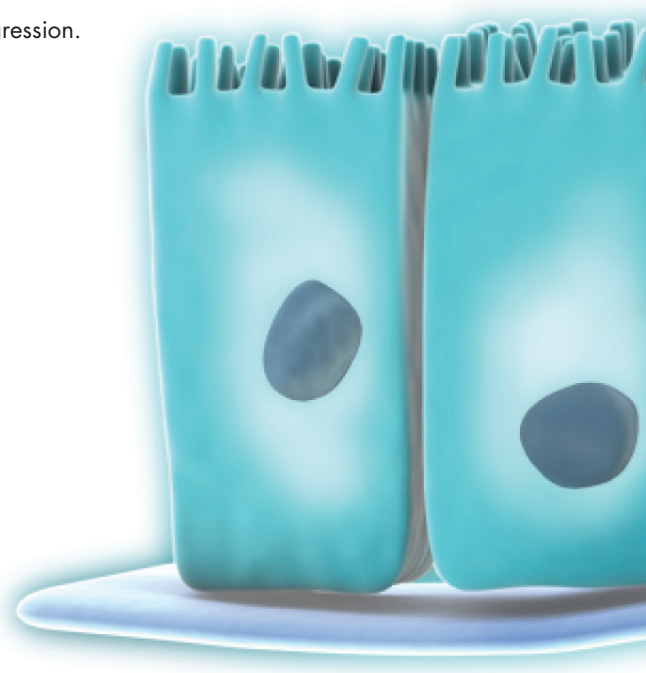


Figure 1. The AdnaTest workflow. The AdnaTest uses a two-step process (Select and Detect) to generate results within 5 hours.

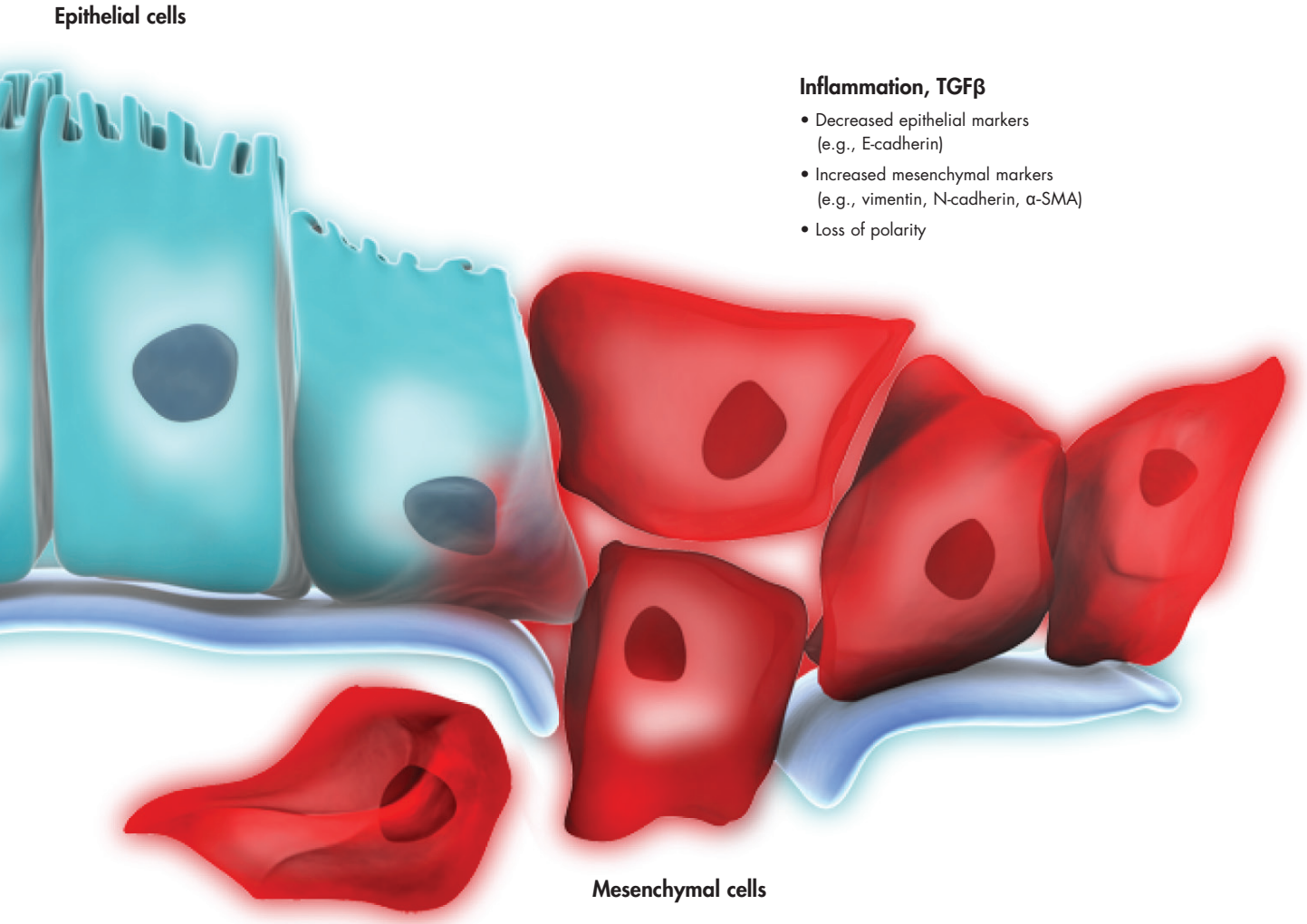


Basement membrane

power of circulating tumor cells

To learn from CTCs, you have to effectively detect and characterize them. That's where the AdnaTest comes in.

The AdnaTest goes beyond the traditional counting of CTCs. The AdnaTest is an open platform to characterize molecular signal transduction pathways. It is a highly specific immunomagnetic cell selection system that combines an optimized antibody mixture with highly sensitive RT-PCR technology. A combination of mRNA tumor markers ensures maximal specificity and sensitivity for CTC detection. The AdnaTest not only enables you to sensitively detect and characterize CTCs and EMT, but also can help you discover new cancer biomarkers.



Get the highest analytical sensitivity a

The AdnaTest provides superior CTC and EMT detection with high analytical sensitivity (fewer false negatives) and high analytical specificity (fewer false positives). You get excellent CTC detection because the AdnaTest features:

- Lower background signals
- A combination of antibodies
- A combination of tumor markers

Lower background signals

Detection of CTCs in a background of normal cells in whole blood challenges you on several levels. One of these is the illegitimate transcription of tumor-associated mRNA by normal nucleated blood cells. Approximately one in a thousand nucleated blood cells produces "illegitimate" or "permissive" tumor-associated mRNAs – leading to a high background signal. Since this background signal can vary from sample to sample, it may be difficult for you to distinguish between positive and negative samples. Lowering these background levels can be achieved by either selectively enriching tumor cells or removing other nucleated cells in the blood. The AdnaTest enables highly specific tumor cell enrichment, using a combination of three different antibodies that bind to different tumor cell epitopes – the regions of the antigen to which the immune system responds. The resulting tumor cell enrichment lowers background signals and increases the AdnaTest's analytical sensitivity for detection of CTCs.

Combination of antibodies

The AdnaTest's use of antibody combinations can increase analytical sensitivity to CTCs by lowering background signals. These antibody combinations serve an additional purpose, increasing analytical specificity by addressing tumor cell variability.

Surface antigen expression on tumor cells is highly variable, and it is essential that the variability of tumor cell expression is also addressed in RT-PCR. Other systems on the market only use one antibody, but AdnaTest Select technology is based on magnetic beads that are coupled to a mixture of antibodies. These mixtures are optimized combinations of antibodies, designed to capture CTCs in their variable expressions, providing greater analytical specificity compared to systems that use only one antibody.

nd specificity for CTC detection

Ensuring specific CTC detection

All AdnaTests guarantee an analytical specificity of at least 90% and a detection limit of two CTCs in 5 ml of blood. An example of analytical specificity and recovery using the AdnaTest BreastCancerSelect and AdnaTest BreastCancerDetect in healthy donors or in spiking experiments is shown in Tables 1 and 2.

Table 1. CTC recovery

Breast cancer	2 cells	5 cells
Positive	159	168
Negative	16	3
Total	175	171
Recovery	**91%**	**98%**

Table 2. CTC specificity

Breast cancer	Healthy donors
Positive	7
Negative	226
Total	233
Analytical specificity	**97%**

Combination of tumor markers

Despite the high analytical sensitivity of the PCR, the determination of a single marker (e.g., CK19) may lower analytical sensitivity. Your chosen marker of interest is not necessarily expressed in every sample. This hit-or-miss approach in searching for CTCs can lead to unpredictable expression patterns, which may lead to false-negative results.

The AdnaTest minimizes this limitation by using a combination of tumor markers at the mRNA level – rather than using a single marker. This important modification greatly improves sensitivity by reducing false-negative results.

The key to sensitive CTC detection: multiple combinations

The AdnaTest's successful CTC detection is based on The Combination of Combinations Principle (COCP). Each AdnaTest has a unique combination of tumor-associated markers and an optimized combination of antibodies for cell selection.

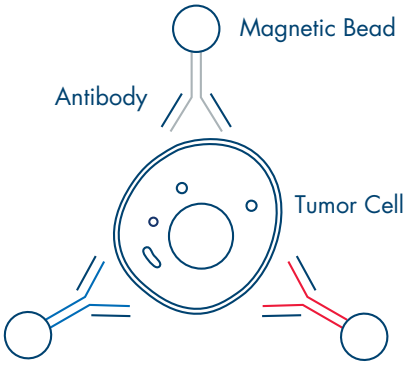


Figure 2. A CTC captured by three antibodies coupled to magnetic beads.

Different target gene combinations used for downstream PCR detection are shown in Table 3. The combination of highly specific immunomagnetic cell selection system (using the optimized antibody combination) and highly sensitive RT-PCR technology (using a combination of mRNA tumor markers) provides the highest degrees of analytical specificity and sensitivity.

Table 3. Target combination

AdnaTest	Target genes
AdnaTest BreastCancer	*MUC1, HER2, EPCAM, ER/PR*
AdnaTest ProstateCancer	*PSA, PSMA, EGFR, AR*
AdnaTest ER/PR	*ER, PR*
AdnaTest ColonCancer	*EPCAM, EGFR, CEA*
AdnaTest OvarianCancer	*EPCAM, MUC1, CA125, ERCC1*
AdnaTest EMT-2 / StemCell	*ALDH1, PI3K, AKT2, Twist* + choice of Breast, Colon, Prostate or Ovarian PCR

Stay flexible – discover new cancer biomarkers

In addition to providing sensitive CTC detection, the AdnaTest's open-ended design enables broad, custom use of the CTC-derived cDNA. This feature lets you analyze and identify molecular pathways in cancer research, so you can identify new, relevant biomarkers. Using the AdnaTest, researchers have already identified CTCs overexpressing AR-V7 in prostate cancer, ERCC1 in ovarian cancer and *HER2* in breast cancer.

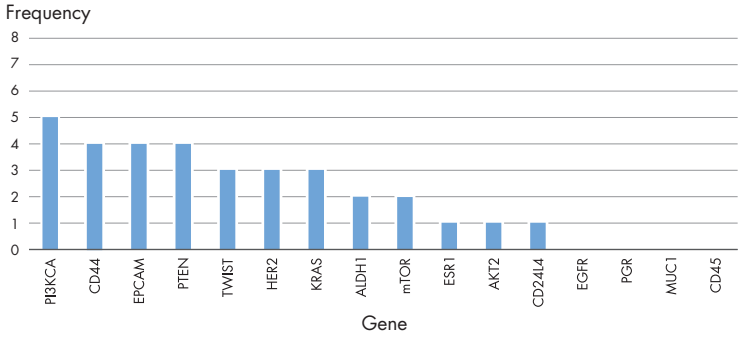


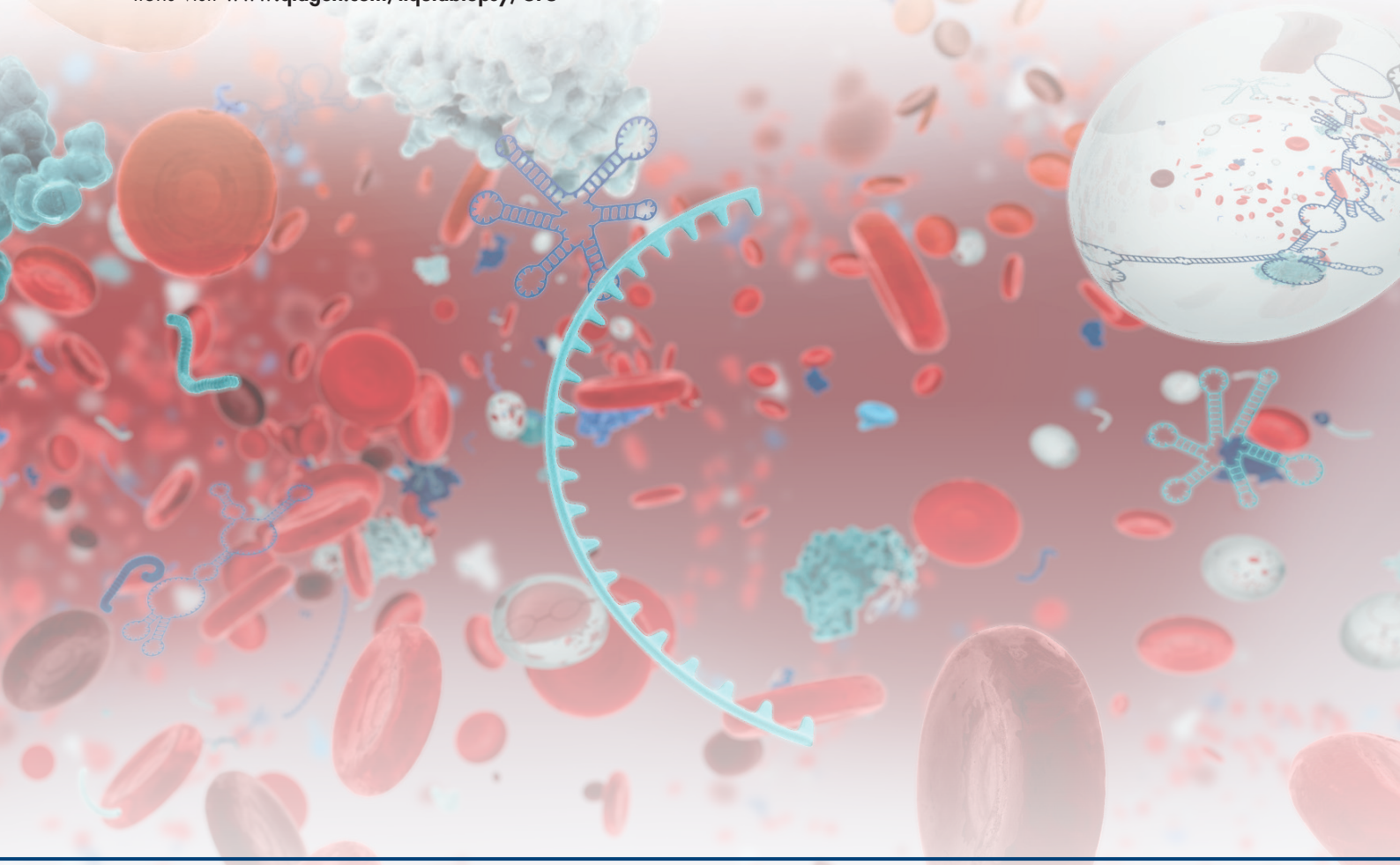
Figure 3. Expression profile of 16 genes in CTCs using AdnaTest EMT2/StemCell. CTC samples from 18 metastatic breast cancer cases were analyzed using the AdnaTest EMT2/StemCell for CTC enrichment and cDNA synthesis, followed by a preamplification step and subsequent qPCR. Genes involved in epithelial characteristics (*EPCAM, CD24L4*), tumor stem cell phenotypes (*CD44, ALDH1*) and epithelial to mesenchymal transition (EMT) via activation of the PI3KCA pathway (*PI3KCA, AKT2, PTEN, mTOR, TWIST*) were detected. In addition, the profiling of breast cancer relevant receptors (*ESR1, PGR, HER2*) was also possible in parallel.

You seek answers – AdnaTest helps you find them!

What can CTCs tell you? Explore with the AdnaTest and find out for yourself!

Molecular characterization of CTCs continues to provide important information for cancer research and for identification of new, relevant biomarkers. New insights into the biology of tumor cell dissemination and dormancy open up new opportunities to improve cancer research.

For ordering information and to find the latest webinars and publications visit **www.qiagen.com/liquidbiopsy/CTC**



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