

THYMIDINE KINASE 1 IN THE STUDY OF SOLID TUMORS



Thymidine Kinase 1 (TK1) has been long known as a valuable biomarker of cellular proliferation. However, previous methods have been based on enzyme activity measurements that may be subject to interference and may underestimate the forms of TK1 found in the serum of subjects with solid tumors.

The AroCell TK 210 ELISA is a new valuable biomarker for studying the rate of proliferation and cell turnover of solid tumors. It brings the specificity of immunoassay to this field and offers improved accuracy when studying serum TK1 derived from patients with solid tumors.

The AroCell TK 210 ELISA will provide new opportunities for studying cellular proliferation, tumor cell turnover and therapy response in subjects with solid tumors.

Thymidine Kinase 1 in Studying Solid Tumors¹

Tissue TK1

DNA replication requires the production of phosphorylated thymidine. There are two enzymes that can perform this, Thymidine Kinase 2 (TK2), found in mitochondria, which is constitutively expressed and Thymidine Kinase 1 (TK1) the expression of which varies during the cell cycle. TK1 concentrations in the cell are low in the G₀/G₁ phase (resting phase) of the cell cycle but increase during the S/G₂ phases when DNA synthesis occurs and then decrease during mitosis². The presence of TK1 in cells is an indicator of active cellular proliferation.

TK1 up-regulation may be an early event in cancer development and TK1 may even be elevated in pre-cancerous conditions³. Increased TK1 expression is often associated with increased expression of cell proliferation markers such as the Ki-67 antigen and proliferating cell nuclear antigen (PCNA)^{4,5,6} although studies have shown that TK1 may be a more useful as a proliferation marker than either of them⁷. TK1 has the further practical advantage in that it is measurable in both tissues and serum, simplifying serial testing.

Increased cellular proliferation is a hallmark of malignancies and TK1 has been found to be significantly over-expressed compared to normal tissue in many solid tumors including lung, colon, prostate, esophagus, stomach, liver, and renal cancers¹.

TK1 activity in serum

95% of serum TK1 activity in cancer patients seems to be tumor derived, making TK1 an excellent indicator of cellular proliferation⁸ and a useful complement to immunohistological testing for proliferation biomarkers. TK1 enzyme activity has been shown to be elevated in subjects with many forms of cancer, including leukaemia, lymphoma, prostate, breast, lung, sarcoma and colon cancer patients¹.

Some studies found that, together with that of traditional tumor biomarkers (e.g. CA 15-3), TK1 activity provided an indication of the rate of cell proliferation and cell turnover, while other markers were related to tumor mass.

Elevations in serum TK1 may be an early event and subjects can have elevated serum TK1 at presentation and high pre-treatment TK1 activities have been associated with shorter progression-free and overall survival in subjects with breast cancer⁹. Conversely, subjects with cancer but lower serum TK1 values may have improved survival¹⁰. Continued elevations in serum TK following surgery may indicate the residual tumor and an increased likelihood of relapse¹¹.

Furthermore, TK1 activity may aid in the selection of therapeutic alternatives in that subjects with high TK1 serum levels may respond better to chemotherapy while those with lower levels may respond better to more specific, e.g. anti-hormonal, therapy¹².

TK1 serum activity is a valuable biomarker but, being an enzyme activity assay, it has limitations regarding specificity and sensitivity in that serum contains inhibitors of TK1 activity and that serum contains multiple forms of TK1 with differing specific activities.

The development of immunoassays for TK1 protein mass can reduce these problems and increase the diagnostic value of TK1 as a biomarker.

TK 210 as a cell proliferation biomarker

As mentioned above, although TK1 activity is a valuable biomarker it has limitations in that most current methods are based on enzyme activity assays¹³. The AroCell TK 210 ELISA test, based on two monoclonal antibodies specific for a unique TK1 amino acid sequence (194-225), brings the specificity, sensitivity and robustness of immunoassay to the assay of serum TK1¹⁴.

Studies at AroCell demonstrated that certain TK1 forms that can be found in subjects with solid tumors differ from those found in normal serum and haematological malignancies. These forms differed in both molecular weight and specific activity and by also measuring these forms the TK 210 immunoassay offered greater discrimination and sensitivity. Furthermore, the use of ELISA simplifies the assay procedure and avoids the effects of interfering substances.

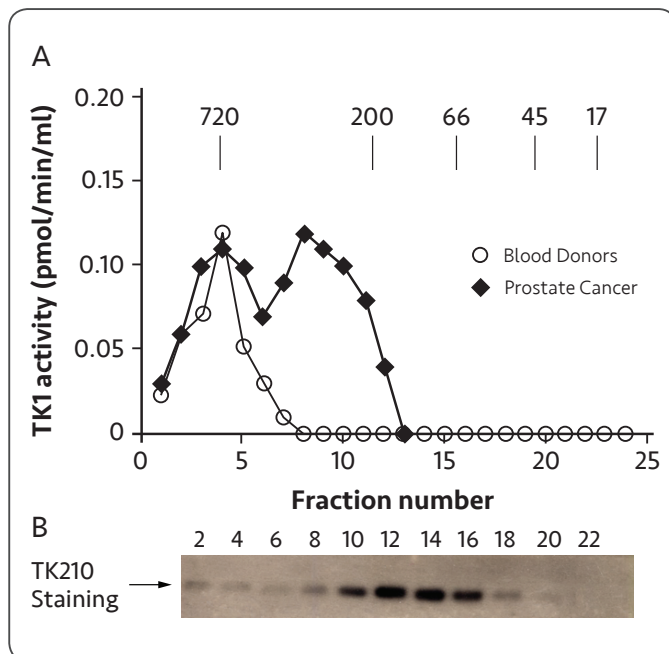
The AroCell TK 210 ELISA test enables TK1 protein levels in subjects with solid tumors to be more clearly distinguished from those found in healthy subjects.



Analysis of Serum TK1 Protein Levels with TK 210-Immunoassay

Greater sensitivity and specificity for tumor associated TK1

Figure 1



TK1 is formed of four identical 25kDa units but serum TK1 enzyme activity is found associated with proteins covering a wide range of molecular weights and this distribution differs between healthy subjects and those with malignancies¹⁵. Figure 1A shows how the distribution of TK1 activity in serum from a subject with prostate cancer differs from that in serum from a healthy blood donor. In the blood donor serum, TK1 activity was found as a single peak of MW between 500-720 kDa whereas in prostate cancer sera, TK1 activity eluted as a broad peak in fractions corresponding to the MW range 200-720 kDa.

Western blot analysis of serum from a prostate cancer subject (figure 1B), using the TK 210 antibody as a probe, showed that TK1 units (25 kDa) were found in fractions corresponding to a wide range of molecular weights, especially below 500 kDa where enzyme activity was low or absent from blood donor sera. Furthermore, there are low molecular weight TK1 fractions (< 200kDa) in serum from prostate subjects where TK1 activity is totally lacking (fractions 14 onward in figure 1B). These results, and similar data from breast cancer, indicate that there are TK1 protein fractions in serum from subjects with solid malignancies which are enzymatically inactive but can be detected and measured immunologically.

TK 210 is more discriminating in solid tumors

TK 210 concentration and TK1 activity in breast cancer

Figure 2

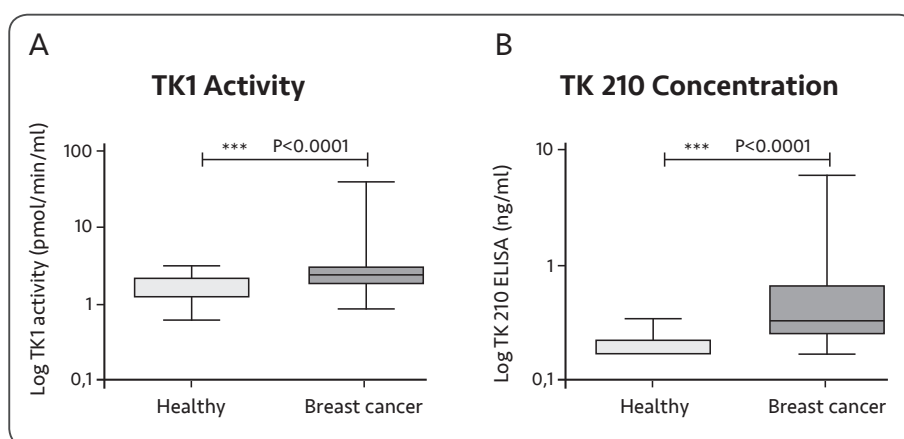
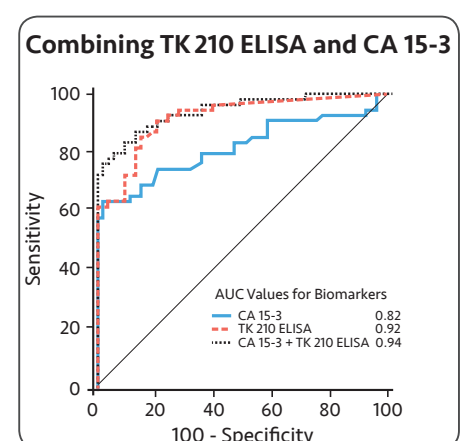


Figure 2 compares TK1 activity levels and TK 210 levels in subjects with breast cancer and blood donors. Note that mean TK 210 ELISA levels differ between breast cancer subjects and blood donors by a factor of five, while TK1 activity levels differ by only a factor of three. Comparing the TK1 activity and TK 210 ELISA as Receiver Operator Characteristic (ROC) curves (Figure 3) shows the greater predictive power of TK 210 ELISA (Area under the curve (AUC) 0.92

Figure 3



v 0.82). Interestingly, both showed greater power than the classic tumor biomarker CA15-3 (0.82). Combining TK 210 ELISA and CA15-3 increased the diagnostic power even further to give an AUC value of 0.94. Sensitivity was increased to 0.76 while specificity was retained (0.98). This shows the potential additive value of TK210 to existing biomarker panels¹⁴.

The AroCell TK 210 ELISA test as a biomarker for solid tumors

- TK1 is an early biomarker of proliferation
- Serum levels reflect tumour proliferation
- TK1 can be used to study many tumors
- Simple ELISA procedure
- Better discrimination between healthy controls and subjects with solid tumors
- Unaffected by serum inhibitors
- TK1 may aid in prognoses and therapy selection

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