

## DiviTum™ – determining pathological cell proliferation in solid tumors from serum samples

### Introduction

DiviTum™ is a new, highly sensitive assay for measuring cell division activity by serum thymidine kinase (s-TK) quantitation. s-TK has been used in Europe and Japan since the 80's for monitoring disease progression and therapy results in lymphomas and leukemia. The increased sensitivity compared to older methods has proved the assay to be useful also for monitoring disease progression and therapy results in solid tumors as ten times lower levels of s-TK activity from tumors can be resolved with DiviTum™ compared to older assays. DiviTum™ has CE label.

This document is intended to give a brief introduction of the product and addresses the biochemical background, the clinical relevance, the product itself, Biovica and applications for a clinical laboratory.

### Biochemical background

Cell division requires DNA duplication and this requires synthesis of building blocks, e.g. thymidine-triphosphate (TTP). In the DNA synthesization, the dividing cell expresses enzymes normally not present, e.g. thymidine kinase (TK). Thymidine kinases (ATP:thymidine-5'phosphotransferase: EC 2.7.1.21) are enzymes that convert deoxythymidine (Thd) to thymidine mono-phosphate (TMP). This phosphorylation is the only pathway to introduce Thd into DNA metabolism. Please refer to Fig. 1 and its caption for the biochemical pathways in the synthesis and catabolism of pyrimidines.

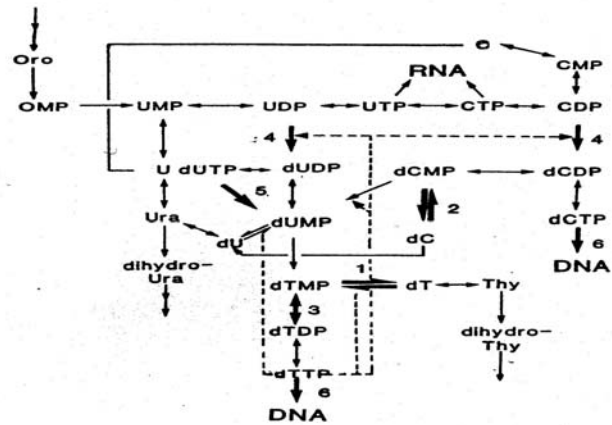
All humans express TK in the body during normal cell division but it is not released to the blood in active form. On the other hand, all cancer types have been found to release enzymatically active TK to the blood stream.

Thus the s-TK activity reflects the total amount of dividing tumor cells in the human body, and this is the single most important parameter for deciding type of clinical intervention and monitoring therapy progression in the oncology field.

For a thorough overview of the biochemical background and clinical application of thymidine kinase, please refer to the review articles by Dr. Gronowitz *et al.* ([www.biovica.com/thymidine1.htm](http://www.biovica.com/thymidine1.htm)).

### Clinical relevance

Transiently increased s-TK levels are observed for certain patient conditions such as acute infection or during wound healing. These levels normalize within a few weeks. Permanent increased TK levels are observed for patients with autoimmune diseases and for cancer patients, and for this group, the change in s-TK tracks the development of the cancer.

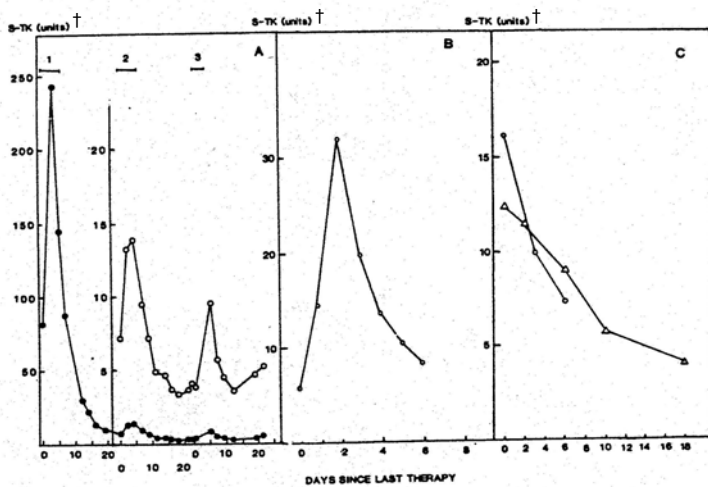


**Fig. 1:** The thymidine triphosphate (TTP) inhibition is shown with dotted lines. Numbers indicate pathways which are catalyzed by proliferation associated enzymes: 1 Thymidine kinase, 2 deoxycytidine kinase, 3 thymidylate kinase, 4 ribonucleotide diphosphate reductase, 5 deoxyuridine triphosphate nucleotide hydrolase

TK measurements have a long and well documented application in hematological diseases for prognosis and therapy follow-up (1–23). For solid tumors, pathological TK levels are connected to metastasized, therapy demanding diseases, and have already been shown to be useful for treatment monitoring (24–34). All above-mentioned studies have used the older technique for TK determination with its limited sensitivity (Prolifigen® TK-REA). The increased sensitivity (by a factor of ten) with the DiviTum™ assay opens up new applications both in the area of therapy monitoring (where therapy can be followed to smaller residual diseases) as well as by detecting primary and relapsing tumor disease at an earlier stage. We also have initial clinical evidence that s-TK levels can indicate whether it is motivated for a person to undergo certain other, more expensive, diagnostic tools such as medical imaging, i.e. DiviTum™ could potentially be used for pre-screening purposes.

## Therapy monitoring

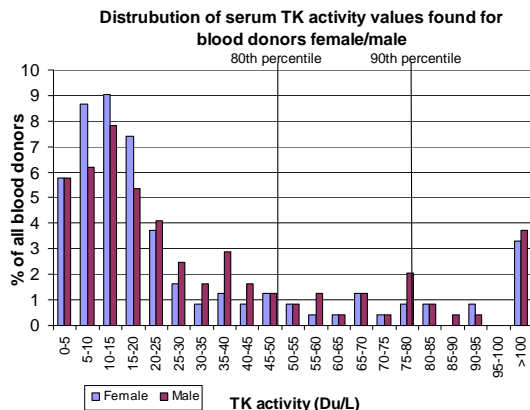
Therapeutic intervention inhibiting cell division results in a rapid decrease of serum TK levels with a half-life of about two days. After a possible s-TK induction peak, from surgery or other treatment, s-TK quickly drops if the therapy has been efficient (Fig. 2, figure taken from the presentations under [www.biovica.com/thymidine1.htm](http://www.biovica.com/thymidine1.htm)).



**Fig 2.** Immediate effect on the s-TK level of different types of therapy. **A)** The s-TK of a patient with acute myeloid lymphoma treated with intermittent POCAL (1, 2, 3 indicates periods with treatment). **B)** The s-TK of a patient with small cell lung cancer treated with a combination of doxorubicin (alternating with CCNU), cyclophosphamide, vincristine and methotrexate. Similar induction peaks were also obtained in colon cancer patients treated with fluorouracil and methotrexate. **C)** The s-TK of a patient with hairy cell leukemia treated with interferon and the s-TK of a patient with prostatic carcinoma treated with Estracyte.

†) Old units from the old test, must be multiplied by 70 to be comparable to DiviTum ng/l values.

Unlike previous thymidine kinase assays, DiviTum™ has the ability to resolve ten times lower s-TK levels from tumors, enabling the lab or treating physician to track the treatment down to normal levels and thus minimize the risk for relapse. Normal levels are shown in Fig. 3. Our tests on blood donor samples have established normal s-TK levels to be below 80 Du/L (DiviTum™-units). The older, radioactive test (also invented by Dr. Gronowitz) could not well resolve tumor signals below ~500 Du/L and thus rendered it less useful for applications outside the hematological area.



**Fig. 3.** Samples obtained from blood donors to determine normal TK activity value. No follow-up was made as to whether patients had slow growing cancer or not (closed study). The study population age was equally distributed between 18 and 80 years.

Observations:

1. No statistical difference across the sexes.
2. 80% of population have TK activity level <50 Du/L.
3. 90% of population have TK activity level <80 Du/L.

## **Pre-screening**

Since only tumors of a certain size are detectable by medical imaging (mammography for breast carcinoma, computer tomography for liver cancer, etc) a promising application area (with the potential to significantly reduce cost of care and diagnosis lead-time and thus to save lives) is pre-screening. In our prostate study (where all enrolled patients were PSA-positive) we found that no patient with a s-TK < 100 Du/L had a positive bone scintigraphy examination. Analogue studies for other expensive diagnostic tools are commenced.

## **Prognosis and therapy selection**

For a given tumor type, TK reflects spread and aggressiveness and thus helps in determining prognosis and therapy selection. For instance, our initial retrospective clinical study on renal carcinoma showed that patients with s-TK above 160 Du/L before nephrectomy had a 90% risk of relapse within 5 years (to be published at the annual meeting of the European Society of Medical Oncology in September 2008). The interpretation is that the nephrectomy failed to remove all of the cancer since it had metastasized and that this metastasis could potentially have been detected beforehand had a cell proliferation test as sensitive as DiviTum™ been available at that time. With the knowledge that a cancer is metastasized, adjuvant therapy can be inserted at an early stage to improve patient prognosis.

## ***DiviTum product***

As previous TK-assays have had a limited sensitivity and the handling of them has been complicated and not possible to automate, TK determination has been made only in specialized laboratories. The easy and standardized procedure of the DiviTum™ assay makes it suitable for general use in clinical laboratories, particularly as it is run in standard 96 well microtiter plate format in an open system. The reagents are stable and the shelf life is long.

DiviTum™ measures TK activity by thymidine incorporation. The quantitation of thymidine kinase activity is difficult as both substrate and product are small soluble molecules. Thus the TK product (TMP) is concomitantly converted to TTP, which is immediately immobilized by DNA-polymerization using a solid-state template in a microtiter plate. The amount of DNA produced is determined by standard ELISA procedure and directly proportional to the TK activity.

In short, the arguments for performing thymidine kinase determination with DiviTum™ can be summarized as follows:

- TK represents an excellent target for early detection of tumor activity and for monitoring therapy results
- DiviTum™ is a new, highly sensitive and reliable way of monitoring thymidine kinase activity and is the ultimate tool on the market for whole body quantitation of pathological cell division
- DiviTum™ is non-radioactive and has 10 times higher sensitivity than Prolifigen™ TK-REA

For the Patient and her/his relatives DiviTum™ provides:

- Personalized care and treatment leading to a better quality of life

For the Medical service organizations DiviTum™ provides:

- A cost effective non-radioactive in-vitro diagnostics test
- A test which does not require extensive investments in facilities and equipment
- A test more easily disseminated, also to small units with less technical capabilities
- A test with potential for screening purposes

For the Laboratory DiviTum™ provides:

- An open format test, simple to perform, easily automated with standard laboratory robotic equipment
- A robust, consistent, non-radioactive IVD test system with high reproducibility
- A possibility to offer pharma and pre-clinical research institutions a unique service in clinical trials, where the effect of experimental therapies on pathological cell proliferation activity can be accurately determined

For the Treating physician DiviTum™ provides:

- A test which detects recurring metastases earlier
- A test which gives a true picture of treatment efficacy (i.e. detects drug resistance effectively) and thus provides for personalized medicine

## **About Biovica and contact information**

Biovica AB (Uppsala, Sweden, [www.biovica.com](http://www.biovica.com)) was founded in 2004 and has developed a new, patented technology for high sensitivity measurements of thymidine kinase activity in body fluids. The DiviTum™ assay is CE labeled and performance validated, both for manual determination and for semi-automated determination on the BioTek ELISA robot. The company has performed initial retrospective clinical studies on sera from breast cancer patients, kidney cancer samples and prostate cancer patients.

If you are interested in evaluating the DiviTum kit in disease progression/regression monitoring or learn more about the product and the results of our initial clinical trials, please contact us at the contact information provided below.

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## **References**

### **Published studies on the use of TK determination in hematological malignancies**

1. Votava T, Topolcan O, Holubec L Jr, Cerna Z, Sasek L, Finek J, Kormunda S. *Changes of serum thymidine kinase in children with acute leukemia*, Anticancer Res. 2007 Jul-Aug; 27(4A):1925-8.
2. Seiler T, Dohner H, Stilgenbauer S. *Risk stratification in chronic lymphocytic leukemia*, Semin Oncol. 2006 Apr; 33(2):186-94. Review.
3. Montillo M, Hamblin T, Hallek M, Montserrat E, Morra E. *Chronic lymphocytic leukemia: novel prognostic factors and their relevance for risk-adapted therapeutic strategies*, Haematologica. 2005 Mar; 90(3):391-9. Review.
4. Madewell BR. *Serum thymidine kinase activity: an alternative to histologic markers of cellular proliferation in canine lymphoma*, J Vet Intern Med. 2004 Sep-Oct; 18(5):595-6.
5. Magnac C, Porcher R, Davi F, Nataf J, Payelle-Brogard B, Tang RP, Opezzo P, Levy V, Dighiero G, Ajchenbaum-Cymbalista F. *Predictive value of serum thymidine kinase level for Ig-V mutational status in B-CLL, Leukemia*. 2003 Jan;17(1):133-7.

6. Di Raimondo F, Giustolisi R, Lerner S, Cacciola E, O'Brien S, Kantarjian H, Keating MJ. *Retrospective study of the prognostic role of serum thymidine kinase level in CLL patients with active disease treated with fludarabine*, Ann Oncol. 2001 May; 12(5):621-5.
7. Hallek M, Langenmayer I, Nerl C, Knauf W, Dietzfelbinger H, Adorf D, Ostwald M, Busch R, Kuhn-Hallek I, Thiel E, Emmerich B. *Elevated serum thymidine kinase levels identify a subgroup at high risk of disease progression in early, nonmolding chronic lymphocytic leukemia*, Blood. 1999 Mar 1; 93(5):1732-7.
8. Poley S, Stieber P, Nussler V, Pahl H, Fateh-Moghadam A. *Serum thymidine kinase in non-Hodgkin lymphomas with special regard to multiple myeloma*, Anticancer Res. 1997 Jul-Aug; 17(4B):3025-9.
9. Poley S, Stieber P, Nussler V, Pahl H, Fateh-Moghadam A. *Evaluation of serum neural cell adhesion molecule as a prognostic marker in multiple myeloma*, Anticancer Res. 1997 Jul-Aug; 17(4B):3021-4.
10. Hallek M, Wanders L, Ostwald M, Busch R, Senekowitsch R, Stern S, Schick HD, Kuhn-Hallek I, Emmerich B. *Serum beta(2)-microglobulin and serum thymidine kinase are independent predictors of progression-free survival in chronic lymphocytic leukemia and immunocytoma*, Leuk Lymphoma. 1996 Aug; 22(5-6):439-47.
11. Sadamori N. *Clinical and biological significance of serum tumor markers in adult T-cell leukemia (Review)*, Leuk Lymphoma. 1996 Aug; 22(5-6):415-9.
12. Aul C, Germing U, Gattermann N, Sohngen D, Heyll A. *The prognostic significance of serum thymidine kinase in the myelodysplastic syndrome (German)*, Dtsch Med Wochenschr. 1996 Sep 13; 121(37):1113-8.
13. Musto P, Bodenizza C, Falcone A, D'Arena G, Scalzulli P, Perla G, Modoni S, Parlatore L, Valvano MR, Carotenuto M. *Prognostic relevance of serum thymidine kinase in primary myelodysplastic syndromes: relationship to development of acute myeloid leukaemia*, Br J Haematol. 1995 May; 90(1):125-30.
14. Rehn S, Gronowitz JS, Källander CFR, Sundström C, Glimelius B. *Deoxythymidine kinase in the tumour cells and serum of patients with non-Hodgkin lymphomas*, Br J Cancer. 1995 May; 71(5):1099-105.
15. Suki S, Swan F Jr, Tucker S, Fritsche HA, Redman JR, Rodriguez MA, McLaughlin P, Romaguera J, Hagemeister FB, Velasquez WS, et al. *Risk classification for large cell lymphoma using lactate dehydrogenase, beta-2 microglobulin, and thymidine kinase*, Leuk Lymphoma. 1995 Jun; 18(1-2):87-92.
16. Musto P, Bodenizza C, Falcone A, D'Arena G, Scalzulli P, Perla G, Modoni S, Parlatore L, Valvano MR, Carotenuto M. *Prognostic relevance of serum thymidine kinase in primary myelodysplastic syndromes: relationship to development of acute myeloid leukaemia*, Br J Haematol. 1995 May; 90(1):125-30.
17. Bogni A, Cortinois A, Grasselli G, Seregni E, Crippa F, Castellani MR, Bombardieri E. *Thymidine kinase (TK) activity as a prognostic parameter of survival in lymphoma patients*, J Biol Regul Homeost Agents. 1994 Oct-Dec; 8(4):121-5.
18. Hallek M, Wanders L, Strohmeyer S, Emmerich B. *Thymidine kinase: a tumor marker with prognostic value for non-Hodgkin's lymphoma and a broad range of potential clinical applications (Review)*, Ann Hematol. 1992 Jul; 65(1):1-5.
19. Doi S, Naito K, Yamada K. *Serum deoxythymidine kinase as a progressive marker of hematological malignancy*, Nagoya J Med Sci. 1990 Mar; 52(1-4):19-26.
20. Martinsson U, Glimelius B, Hagberg H, Sundström C. *Prognostic relevance of serum-markers in relation to histopathology, stage and initial symptoms in advanced low-grade non-Hodgkin lymphomas*, Eur J Haematol. 1988 Apr; 40(4):289-98.

21. Källander CFR, Simonsson B, Gronowitz JS, Nilsson K. *Serum deoxythymidine kinase correlates with peripheral lymphocyte thymidine uptake in chronic lymphocytic leukemia*, Eur J Haematol. 1987 Apr; 38(4):331-7.
22. Källander CFR, Simonsson B, Hagberg H, Gronowitz JS. *Serum deoxythymidine kinase gives prognostic information in chronic lymphocytic leukemia*, Cancer. 1984 Dec 1; 54(11):2450-5.
23. Hagberg H, Glimelius B, Gronowitz S, Killander A, Källander C, Schroder T. *Biochemical markers in non-Hodgkin's lymphoma stages III and IV and prognosis: a multivariate analysis*, Scand J Haematol. 1984 Jul; 33(1):59-67.

## **Published studies on the use of TK determination in solid tumors**

24. Ondrej Topolcan and Lubos Holubec Jr. *The role of thymidine kinase in cancer diseases*, Expert Opinion on Medical Diagnostics, 2008 Feb; 2(2):129-141
25. Svobodova S, Topolcan O, Holubec L, Treska V, Sutnar A, Rupert K, Kormunda S, Rousarova M, Finek J. *Prognostic importance of thymidine kinase in colorectal and breast cancer*, Anticancer Res. 2007 Jul-Aug;27(4A):1907-9.
26. He Q, Fornander T, Johansson H, Johansson U, Hu GZ, Rutqvist LE, Skog S. *Thymidine kinase 1 in serum predicts increased risk of distant or loco-regional recurrence following surgery in patients with early breast cancer*, Anticancer Res. 2006 Nov-Dec;26(6C):4753-9.
27. Zhang J, Jia Q, Zou S, Zhang P, Zhang X, Skog S, Luo P, Zhang W, He Q. *Thymidine kinase 1: a proliferation marker for determining prognosis and monitoring the surgical outcome of primary bladder carcinoma patients*, Oncol Rep. 2006 Feb;15(2):455-61.
28. Li HX, Lei DS, Wang XQ, Skog S, He Q. *Serum thymidine kinase 1 is a prognostic and monitoring factor in patients with non-small cell lung cancer*, Oncol Rep. 2005 Jan;13(1):145-9.
29. Hallek M, Touitou Y, Levi F, Mechkouri M, Bogdan A, Bailleul F, Senekowitsch R, Emmerich B. *Serum thymidine kinase levels are elevated and exhibit diurnal variations in patients with advanced ovarian cancer*, Clin Chim Acta. 1997 Nov 28;267(2):155-66.
30. Letocha H, Eklöv S, Gronowitz JS, Norlen BJ, Nilsson S. *Deoxythymidine kinase in the staging of prostatic adenocarcinoma*, Prostate 1996; 29:15-9.
31. Ekman P, Lewenhaupt A. *Serum tumor markers in human prostatic carcinoma. The value of a marker panel for prognostic information*, Acta Oncol. 1991; 30:173-5.
32. Lewenhaupt A, Ekman P, Eneroth P, Nilsson B. *Tumor markers as prognostic aids in prostatic carcinoma*, Br.J.Urol. 1990; 66:182-7.
33. Gronowitz JS, Bergström R, Nôu E, et al. *Clinical and serologic markers of stage and prognosis in small cell lung cancer. A multivariate analysis*, Cancer 1990; 66:722-32.
34. Gronowitz JS, Steinholtz L, Källander CFR, Hagberg H, Bergh J. *Serum deoxythymidine kinase in small cell carcinoma of the lung. Relation to clinical features, prognosis, and other biochemical markers*. Cancer 1986; 58:111-8.
34. Larson A, Fritjofsson A, Norlen BJ, Gronowitz JS, Ronquist G. *Prostate specific acid phosphatase versus five other possible tumor markers: a comparative study in men with prostatic carcinoma*, Scand.J.Clin.Lab.Invest.Suppl. 1985; 179:81-8.