

## Are you clinically evaluating cancer therapies without directly measuring cell proliferation rate?

**Then DiviTum™ offers a solution!**

**DiviTum™ - quantitating whole body cell proliferation activity using a serum sample**

### Introduction

#### Why measure cell proliferation activity?

The speed at which tumor cells proliferate is one of the most fundamental characteristics of cancer and also the most important factor in determining patient prognosis. It is little surprising that many cancer therapies are targeting cell division mechanisms and that the effect of experimental drugs on cell division is studied in-vitro in cell cultures in many pre-clinical trials. Incorporating thymidine into the DNA is one of the more accurate methods for such cell culture experiments. DiviTum™ now offers the drug development industry the possibility to use this highly accurate method for determining whole body cell proliferation activity in their pre-clinical and clinical studies, both on man and in animal models such as cat and mice. By using the manual or semi-automated DiviTum™ assay and a simple serum sample, the user can assess the effect of a drug on the tumor cell proliferation rate with unpre-cedented sensitivity. DiviTum™ is non-radioactive, has CE label and can be used for research purposes in the US.

#### In your next clinical trial, ask yourself the following:

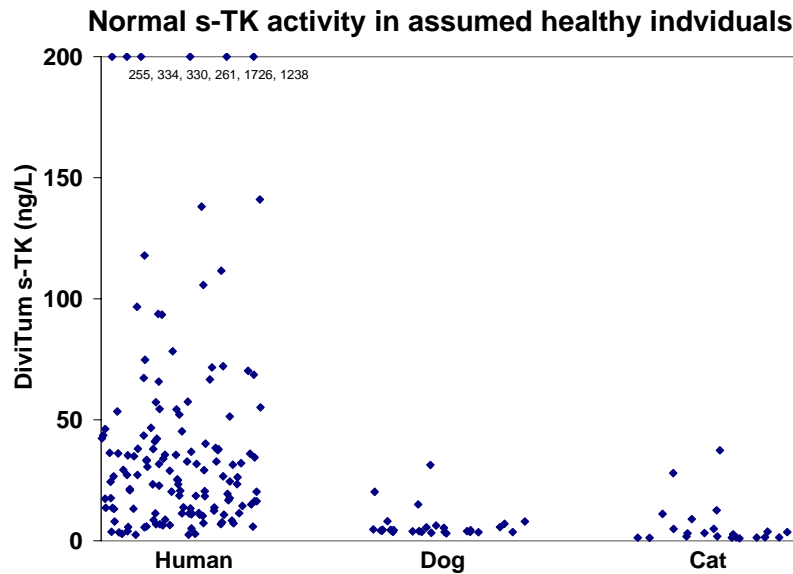
- Is the degree of cell proliferation important for your therapy area?
- Are you interested in quickly being able to determine if a new drug decreases the cell proliferation activity in animal models and in human?
- Are you interested in being able to monitor cell proliferation activity over time with unprecedented sensitivity using a serum sample and a simple assay?

If so, please read this informational material on DiviTum™, our ultra sensitive assay for whole body cell proliferation quantitation based on thymidine incorporation!

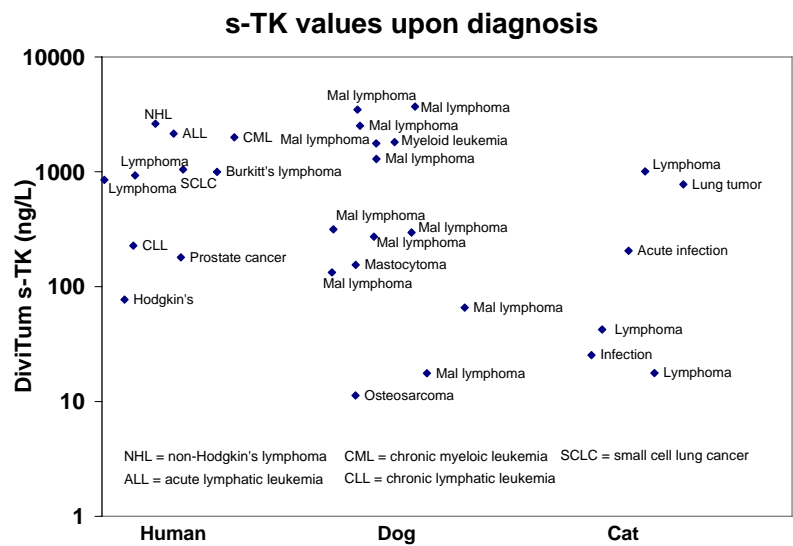
### Background

All humans express thymidine kinase (TK) in the body during cell division and small amounts of active enzyme is released to the blood in healthy individuals (see Fig. 1). The serum thymidine kinase (s-TK) activity is elevated in diseases with increased cell division and the s-TK activity reflects the total amount of dividing tumor cells in the human body. The highest levels, 10.000 times normal, have been found in acute leukemia whereas patients with slow growing prostate cancer can exhibit normal levels for many years. The s-TK level upon detection of a given cancer type relates to stage, spread and malignancy and is thus a strong prognostic factor.

Normal levels for human, dog and cat are shown in Fig. 1. Fig. 2 indicates s-TK levels upon detection in some individuals for some cancer diseases.



**Fig. 1.** Human samples obtained from blood donors, age equally distributed 18 – 80 years. No follow-up was made as to whether patients had undiagnosed cancer or not (closed study). There were no statistical differences across the sexes. All data on dog and cat courtesy of Dr. H. von Euler, head of the center of clinical comparative oncology at the Swedish University of Agricultural Sciences.



**Fig. 2.** s-TK activity upon initial detection of cancer for some selected diseases. For a given tumor type the s-TK activity reflects the growth rate of the cancer.

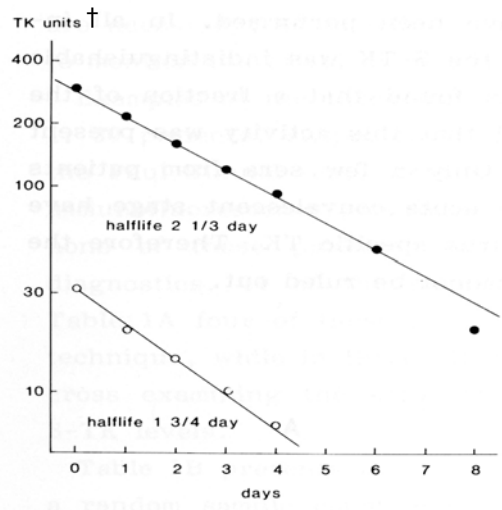
Transiently increased s-TK levels are observed for certain patient conditions such as acute infection but normalize within a few weeks. Permanent increased TK levels are observed for patients with autoimmune diseases and for cancer patients, and for the latter group, the change in s-TK tracks the development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

## Monitoring

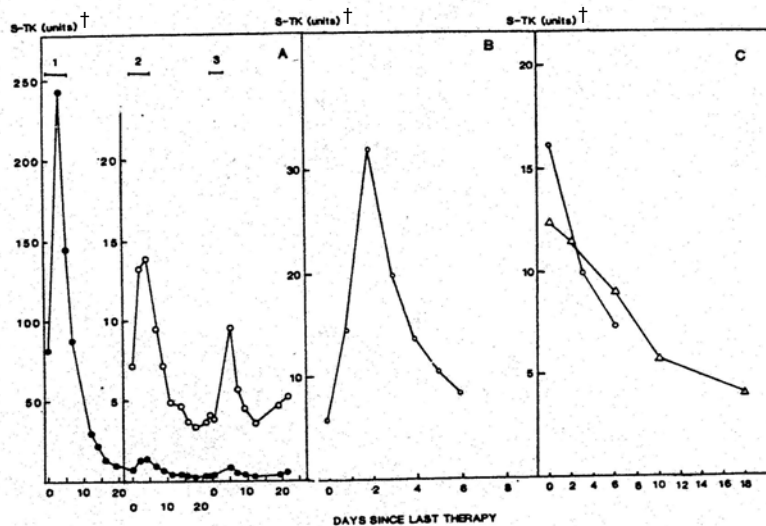
Therapeutic intervention inhibiting cell division results in a rapid decrease of serum TK levels with a half-life of about two days.

**Fig 3.** Half life of s-TK. (●) patient with pernicious anemia after B<sub>12</sub> injection; (○) patient with small cell carcinoma of the lung, decrease of therapy induced s-TK.

†) Old units from old, radioactive test, must be multiplied by 70 to be comparable to DiviTum™.



After a possible s-TK induction peak from treatment (panel A in Fig. 4), s-TK quickly drops if the therapy has been efficient. s-TK has been used in Europe and Japan since the 80's for monitoring disease progression and therapy results in spread cancer disease, mainly for leukemia and lymphoma. In Fig. 4 (unpublished data taken from the presentations under [www.biovica.com/thymidine1.htm](http://www.biovica.com/thymidine1.htm)) the effect of some cancer therapies is monitored over time with s-TK. In Fig. 5, the treatment of a dog is followed down to normal levels.

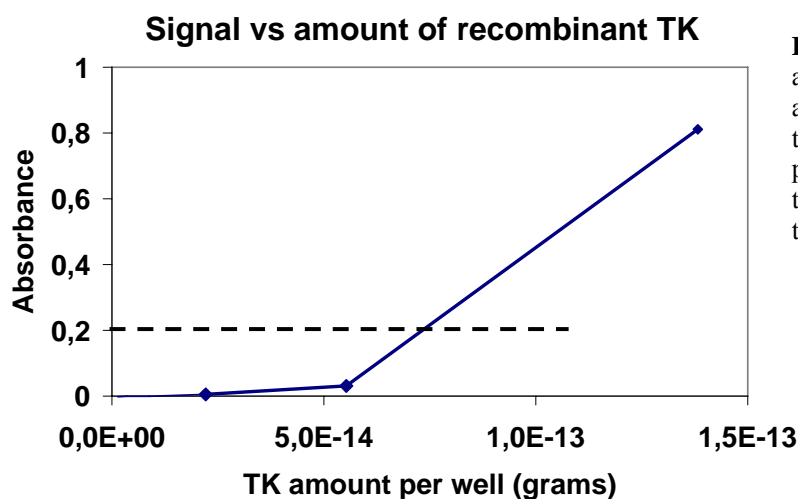


†) Old units from the old test, must be multiplied by 70 to be comparable to DiviTum ng/l values.

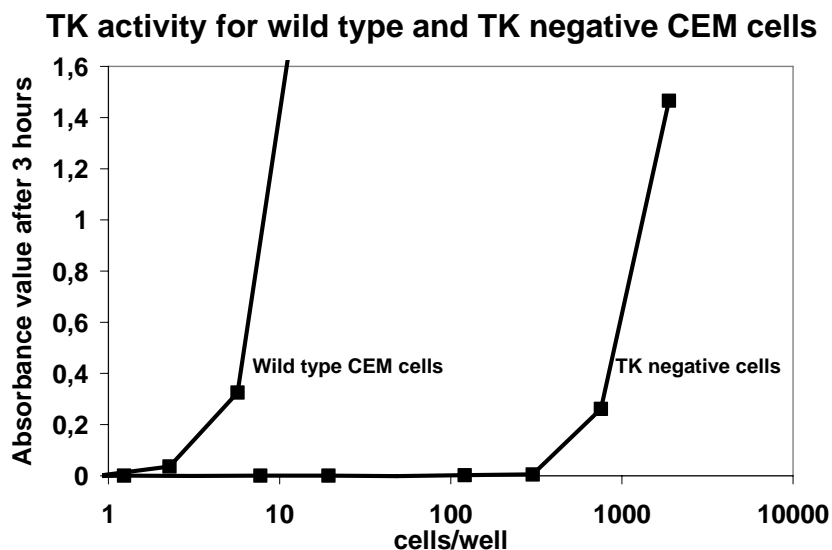
**Fig 4.** Immediate effect on the s-TK level of different types of therapy. **A)** The s-TK of a patient with acute myeloid lymphoma treated with intermittent POCAL (1, 2, 3 indicates periods with treatment). **B)** The s-TK of a patient with small cell lung cancer treated with a combination of doxorubicin (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.

## DiviTum product

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 micro titer plate format in an open system (ELISA). The reagents are stable and the shelf life is long. The below graphs indicate the sensitivity and specificity of the product.



**Fig 5.** DiviTum™ measures TK activity by thymidine incorporation and is so sensitivity that it can detect the TK activity from  $7 \cdot 10^{-14}$  grams TK per well after three hours, as shown by this experiment with re-combinant thymidine kinase.



**Fig. 6.** Serial dilution of wild type and TK negative CEM cells shows that DiviTum™ can measure TK activity from only 5-6 cells per well with reading after three hours. For TK negative cells (still expressing mitochondrial TK) 150 times higher concentrations are needed to get similar signal strength. DiviTum™ is thus highly specific for measurements of thymidine kinase 1 (TK 1).

## About Biovica and contact information

Biovica AB 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 and implemented on the BioTek ELISA robot.

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

Hans Bornefalk, PhD  
CEO  
hans.bornefalk@biovica.com  
Mobile: +46 703 304 816

Simon Gronowitz, Assoc. Prof.  
Founder and head of R&D  
simon@biovica.com  
Mobile: +46 762 418 166

### Biovica AB

Uppsala Science Park  
Dag Hammarskjölds väg 32 B  
SE-751 83 Uppsala, Sweden  
Tel +46 (0)18 57 24 24  
Fax + 46 (0)18 57 24 28  
info@biovica.com  
www.biovica.com