Proteom Services

Material Information Sheet

Polyclonal antiserum against human dUTPase
Produced in rabbit immunized with recombinant human dUTPase protein
To be stored at -80^{0}C

What is it?

Deoxyuridine triphosphate nucleotidohydrolase (dUTPase) is a ubiquitous enzyme that catalyzes the hydrolysis of dUTP to dUMP and pyrophosphate. dUTPase is essential for viability. Its targeting in human cancer has been proposed as a novel promising therapeutic avenue to perturb thymidylate homeostasis. Canman and co-workers have demonstrated that high levels of dUTPase induce reduced sensitivity to the cancer chemotherapeutic agent fluorodeoxyuridine (FUdR), a thymidine synthase inhibitor [1,2]. Based on these findings, estimation of dUTPase levels in cancer cells is considered to be an important measure to predict success of thymidylate-targeted chemotherapy [3,4].
Two distinct forms of dUTPase exist in humans. Cellular fractionation experiments suggest that the more abundant, lower molecular mass (22 kDa) form of dUTPase (DUT-N) localizes in the nucleus, while the higher molecular mass (23 kDa) form (DUT-M) is associated with the mitochondria. The two isoforms differ within the N-terminal region but are identical within a large portion of the C-terminus including the catalytically indispensable domains[5].
To follow cellular dUTPase levels, Western blotting and immuno-histochemistry would offer the most adequate possibilities. The present polyclonal serum is offered for this task.

How to use it?

N.B. Protocols have to be optimized in each laboratory.
Western blotting: In our lab, serum diluted up to 50,000 - 100,000-fold and use of super-sensitive Immobilon ECL western chemiluminescent bloting reagent (Millipore-WBKL S0 100) proved to be optimal.
Immunohistochemistry: use serum at 5,000 - 10,000 fold dilution.
Immunoprecipitation: immobilize IgGs from the serum on protein A agarose. Use the loaded beads according to usual protocols.

How NOT to use it?

This product is for R&D use only, not for drug, household, or other uses. Please observe safe handling practices as usual in molecular biology.

What does it contain?

Serum liquid from rabbits immunized with recombinant human dUTPase protein

Representative Western blotting results (optimized protocol)

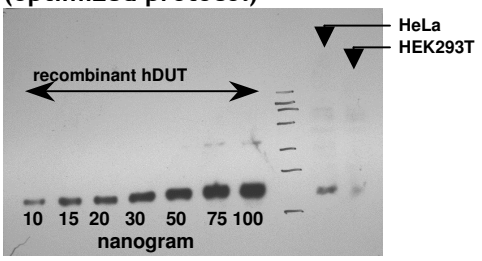

Figure 1. Visualization of reactive protein bands in control and human cell extracts. Similar amounts of total cell protein were loaded. Serum was used at 75,000-fold dilution. Under these circumstances, only the more abundant nuclear isoform is observed in the cell extracts.

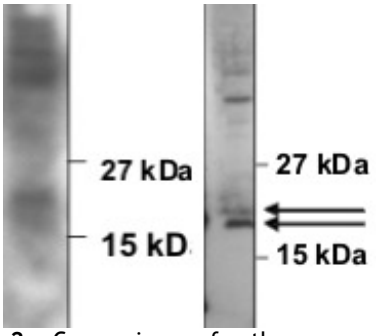

Figure 2. Comparison of other commercially available polyclonal serum (used at 2,000-fold dilution, left object) and the present polyclonal serum (used at 25,000-fold dilution, right object). HeLa cell extracts were used. Double arrows indicate the two isoforms, detectable with the present polyclonal serum. Although some unspecifc bands are visible in both cases, the present serum is clearly superior.

What to read if in doubt?

(1) Canman, C. E. et al. J. *Cancer Res* **1993**, *53*, 5219-24.
(2) Canman, C. E. et al. *Cold Spring Harb Symp Quant Biol* **1994**, *59*, 277-86.
(3) Ladner, R. D. *Curr Protein Pept Sci* **2001**, *2*, 361-70.
(4) Grasser, F. A. et al. *Curr Protein Pept Sci* **2001**, *2*, 349-60.
(5) Ladner, R. D.; Caradonna, S. J. *J Biol Chem* **1997**, *272*, 19072-80.