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# Product Information

**Anti-DHFR, C-Terminal**
produced in rabbit, affinity isolated antibody

Catalog Number **D0942**

## Product Description

Anti-DHFR, C-Terminal is produced in rabbit using a synthetic peptide corresponding to amino acids 171-185 of mouse DHFR, conjugated to KLH via an N-terminal added cysteine residue as the immunogen. The immunizing sequence is conserved in mouse, human and rat. The antibody is affinity purified on the immunizing peptide immobilized on agarose.

Anti-DHFR, C-Terminal reacts specifically with DHFR. Specific staining is inhibited by the DHFR immunizing peptide.

DHFR (Dihydrofolate reductase) is a 187 amino acid enzyme that catalyzes the reduction of folic acid in two NADPH-dependent steps, which first yield 7,8-dihydrofolate (DHF) and then 5,6,7,8-tetrahydrofolate (THF).[1,2] THF is the coenzyme for thymidilate synthetase in the biosynthesis of thymidine, participating in the biosynthesis of amino acids and purines as well. Since folic acid reduction is essential for generating the active coenzyme, DHFR becomes critical in DNA replication.[2,3] For this reason, much research has been done to determine the structure and mechanism of action of the enzyme.[3,4] Inhibitors of DHFR, such as methotrexate, are folate analogs which can bind to the active site and deactivate the enzyme. Methotrexate has important implications for cancer therapy.[5]

In addition, structural and enzymatic properties of DHFR has led to the development of a variety of screening methods in which DHFR functions in a fashion that resembles reporter genes. The screenings are based on the fact that DHFR can be dissected in two halves that can be then reassembled to form an active enzyme.[6] Thus, each of the two halves of DHFR can be expressed as two fusion proteins that when interacting with each other, can restore the DHFR enzymatic activity. The readout of this protein-protein interaction and consequent enzymatic activity can be either restoration of growth in bacterial, yeast, and plant, or receptor activation.[7-11]

The approach can be used for receptor-ligand, antigen-antibody, and other interactions.[8,10,11] Antibodies specific for DHFR are a useful tool for following and identifying such protein interactions.

## Reagent

Supplied as a solution in 0.01M phosphate buffered saline pH 7.4 containing 1% bovine serum albumin and 15 mM sodium azide.

Antibody Concentration: 1.0 -1.5 mg/ml

## Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

## Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

## Procedures

Procedure for Immunoblotting

Note: The whole procedure is performed at room temperature.

1. Separate extracts containing DHFR or DHFR fusion proteins from sample lysates using a standard SDS-PAGE protocol.
   Note: The amount of extract depends on the level of expression of the fusion protein and the specific application.
2. Transfer proteins from the gel to a nitrocellulose membrane.
3. Block the membrane using a solution of PBS containing 5% non-fat dry milk (PBS, Catalog No. D8537; non-fat dry milk, Catalog No. M7409) for at least 60 minutes.

4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20, Catalog No. P3563.
5. Incubate the membrane with Anti-DHFR antibody as the primary antibody in PBS containing 0.05% TWEEN 20, with agitation for 120 minutes
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN 20.
7. Incubate the membrane with Anti-Rabbit IgG-Peroxidase, Catalog No. A0545, as the secondary antibody at the recommended concentration in PBS, containing 0.05% TWEEN 20. Incubate with agitation for 60 minutes. Adjust the product concentration to maximize detection sensitivity and to minimize background.
8. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN 20.
9. Treat the membrane with a peroxidase substrate**.**

Procedure for Immunoprecipitation

1. Centrifuge 40 µL of a 1:1 suspension of Protein A-Agarose beads, Catalog No. P3476, for 1 min. at 12,000x*g*, and then wash twice with 1 ml RIPA buffer (50 mM Tris Base, 0.25% w/v Deoxycholate, 1% NP40, 150 mM NaCl, 1 mM EDTA, pH 7.4) at 4 ºC.
2. Add Anti-DHFR antibody diluted in PBS, and incubate by swinging head-over-tail for 1 hour at room temperature.
3. Centrifuge 1 min 12,000x*g*, wash twice with 1 ml RIPA at 4 ºC.
4. Add 0.1-1.0 ml of cell extract containing DHFR tagged protein to the beads, or purified DHFR (see note), and incubate from 2 hours to overnight at 4 ºC, while swinging head-over tail.
   **Note**: The amount of cell extract depends on the level of expression of the tagged protein and the specific application.
5. Spin down beads; remove supernatant
6. Wash beads four times with 1ml RIPA buffer and once with PBS by vortex and short spin.
7. Resuspend pellet in 25 µl 2XSDS-PAGE sample buffer. Boil sample for 5 min and spin down. The sample is ready to be loaded on an SDS-PAGE gel.

**Product Profile**

Immunoblotting (chemiluminiscent): a working concentration of 0.5-1.0 µg/ml is recommended using 50-100 ng of purified recombinant DHFR, or 293T human embryonic kidney cell extract, or Jurkat human acute T Cell leukemia cell extract

Immunoprecipitation: 0.5-1.0 µg of the antibody can precipitate 100-200 ng of purified DHFR.

**Note:** In order to obtain the best results in various techniques and preparations, we recommend determining optimal working dilutions by titration.

**References**


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