

DESCRIPTION

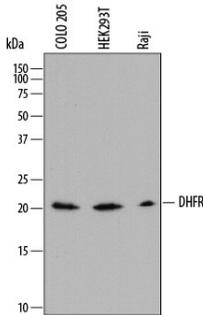
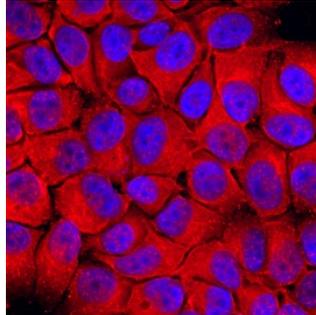
Species Reactivity	Human
Specificity	Detects human Dihydrofolate Reductase/DHFR in direct ELISAs and Western blots.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human Dihydrofolate Reductase/DHFR Met1-Asp187 Accession # P00374
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below

DATA

<p>Western Blot</p>  <p>Detection of Human Dihydrofolate Reductase/DHFR by Western Blot. Western blot shows lysates of COLO 205 human colorectal adenocarcinoma cell line, HEK293T human embryonic kidney cell line, and Raji human Burkitt's lymphoma cell line. PVDF membrane was probed with 0.5 µg/mL of Sheep Anti-Human Dihydrofolate Reductase/DHFR Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7934) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for Dihydrofolate Reductase/DHFR at approximately 21 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunocytochemistry</p>  <p>Dihydrofolate Reductase/DHFR in MCF-7 Human Cell Line. Dihydrofolate Reductase/DHFR was detected in immersion fixed MCF-7 human breast cancer cell line using Sheep Anti-Human Dihydrofolate Reductase/DHFR Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7934) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.</p>
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PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

DHFR (DiHydroFolate Reductase; also Tetrahydrofolate dehydrogenase) is a 21-23 kDa member of the dihydrofolate reductase family of enzymes. It is a ubiquitously expressed monomer, and considered to be a housekeeping gene. Housekeeping genes are those that play a role in multiple pathways, although not the same pathway(s) in all cells. DHFR participates in the reduction of dihydrofolate to tetrahydrofolate, a product that is subsequently used in the synthesis of purines and thymidylic acid that are used to generate both RNA and DNA. Within the cell, DHFR is known to exist in two pools: one contains DHFR bound to its own RNA where it acts as a transcriptional repressor, while another contains DHFR bound to NADPH. Human DHFR is 187 amino acids (aa) in length and possesses one DHFR domain (aa 4-185). Its mRNA binding motif is suggested to involve Cys6, Leu22, Glu30 and Ser118. There is one potential alternative start site found 75 aa upstream of the standard start site. Full length human DHFR (aa 1-187) shares 90% aa sequence identity with mouse DHFR.