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Product Information

ANTI-CHLORAMPHENICOL ACETYL TRANSFERASE (CAT) IgG Fraction of Antiserum

Product No. **C 9336**

Product Description

Anti-CAT is generated in rabbits immunized with Chloramphenicol Acetyl Transferase (CAT).

Applying immunoblotting, the antibody identifies the bacterial CAT or the recombinant CAT expressed in eukaryotic cells (a predominant band around 26 kD). Bacterial CAT antigen inhibits the staining of CAT by the antibody in CAT transfected cell extracts. Assayed by indirect immunofluorescence, the antibody detects eukaryotic cells that are transfected with a plasmid bearing the CAT gene and express CAT in the cytoplasm.

The use of an antibody to detect CAT can provide an alternative non-isotopic CAT detection assay. The antibody can be used to detect CAT protein level and expression within the cell, allowing the study of localization signal sequences.

Analysis of genetic elements required for specific gene expression is usually based on the attachment of these elements to reporter gene in a plasmid. The plasmid is inserted into cells and reporter gene expression is assayed within 48 hours after introduction of DNA. The amount of the reporter protein synthesized under the experimental conditions is presumed to reflect the ability of the sequences studied to direct or promote transcription. CAT has been the most frequently used reporter enzyme. This enzyme catalyzes the transfer of acetyl groups from acetylcoenzyme A to chloramphenicol. CAT is a prokaryotic enzyme, hence there are minimal competing activities in most eukaryotic cells. Therefore, the signal to noise ratio using this system is high. CAT is quite stable, making it suitable as a reporter in transient transfection experiments. The advantage of the system is that the enzyme assays are relatively easy. The main disadvantage is that the enzymatic assay is based on the use of radioactive substrate and the assay can be time consuming.

The alternative non-isotopic CAT detection assay is based on immuno detection of the enzyme using an anti-CAT antibody. This assay directly detects CAT protein level; therefore, it does not require CAT activity.

This allows one to treat the cells with more harsh buffers and eliminates the need for radioactive reagents. Moreover, antibodies can detect CAT expression within the cell, thus providing the means to study localization signal sequences using CAT as a reporter gene.

Reagents

The product is provided as an IgG fraction in 0.01 M phosphate buffered saline pH 7.4, with 15 mM sodium azide as a preservative.

Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Product Profile

Protein Concentration: At least 10mg/ml.

A working dilution of at least 10 µg/ml is determined by immunoblotting on transfected* cell extracts using alkaline phosphatase conjugated secondary antibody.

A working dilution of at least 10 µg/ml is determined by indirect immunofluorescence on methanol-acetone fixed transfected* cells using FITC conjugated secondary antibody.

Transfected with a reporter plasmid containing the gene for CAT.

In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

Storage /Stability

For continuous use, store at 2-8 °C for a maximum of one month. For extended storage freeze in working aliquots. Repeated freezing and thawing is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

References

1. Alam, J. and Cook, J., Anal. Biochem., **188**, 245 (1990)..
2. Gorman, C., et al., Mol. Cell Biol., **2**, 1044 (1982).

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