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Product Information

MONOCLONAL ANTI-CELLULOSE BINDING DOMAIN (CBDclos), CLONE CBD-8 Mouse Ascites Fluid

Product No. **C 5473**

Product Description

Monoclonal Anti-Cellulose Binding Domain (CBDclos) (mouse IgG2b isotype) is derived from the CBD-8 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a recombinant 17 kD fragment of the cellulase complex from *Clostridium cellulovorans*. The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-Cellulose Binding Domain (CBDclos) recognizes CBDclos (CBD family IIIa, from *Clostridium cellulovorans*, 17 kDa). By immunoblotting, the product recognizes the CBDclos tag expressed by the vector pET-34b.

Recombinant DNA technology enables the insertion of specific sequences or genes into the gene of interest, which can provide 'affinity handles' designed to bind specific matrices. The use of these tags enables the selective identification and purification of the protein of interest¹⁻³. However, there are problems encountered when using many of the affinity tags. These problems include the incorrect folding of recombinant molecules (thus masking the ligand active site), and the requirement to cleave off the fusion protein and re-purify the parent protein. An improved purification process has been developed by genetically engineering sequences of tails or tags away from the protein active site. Many affinity purification systems share the common drawback of the high cost of the affinity matrix, and also suffer from relatively slow flow rates and high back-pressure in column chromatography applications. Cellulose Binding Domain (CBD), a protein domain that naturally binds to cellulose, is an unbranched homopolymer of $\beta(1-4)$ linked glucose subunits⁴. CBDs are found in nature as discrete domains in proteins, such as in cellulases, as well as in proteins that have no hydrolytic activity. Over 120 different CBD sequences have been identified and classified into at

least 11 families⁵. The best known families are I through IV, the CBDs of which average 36, 105, 150 and 150 amino acids in size, respectively. Members of the CBD family differ in both their binding and elution properties with different cellulose matrices. It has been reported that the addition of a cellulose binding domain (CBD) tag creates a stable fusion product that does not appear to interfere with the bioactivity of the protein or with the bio-distribution of the CBD tagged product.⁶⁻⁸ For example, sequences of CBDclos (a 17 kDa domain from *Clostridium cellulovorans*), CBDcenA (a 11.7 kDa domain from *Cellulomonas fimi*), and CBDcex (a 10.8 kD domain from *Cellulomonas fimi*), have been incorporated into the widely used pET expression vectors. CBDclos and CBDcenA are located at the N-terminus, while CBDcex is at the C-terminus of the pET vector. The expression of polypeptides in-frame with CBD allows for their easy purification from bacterial extracts under mild conditions and employs a single affinity chromatographic step on cellulose resin. Cellulose is an attractive matrix for affinity purification and immobilization, mainly due to its excellent physical properties and low price. It is commercially available in many different forms, such as cotton wool, filters, beads, powders, fibers, hydrogel, membranes, and sheets of defined porosity. The CBD system incorporates cleavage sites that separate CBD from its partner protein⁹. Indeed, many recombinant proteins have been engineered with CBD tags to facilitate the detection, isolation and purification of the proteins. A monoclonal antibody reacting specifically with CBDclos may be useful in various immunotechniques to identify the expression of a CBDclos fusion protein in bacteria, bacterial lysates, or cells and tissues transfected with a CBDclos fusion protein expressing vector.

Reagents

The product is supplied as ascites fluid with 15 mM sodium azide as a preservative.

Precautions and Disclaimer

Due to the sodium azide content a material safety 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.

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 is not recommended.

Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile

A minimum working dilution of 1:20,000 is determined by immunoblotting using a recombinant 17 kDa fragment of the cellulose complex from *Clostridium cellulovorans*.

Note: In order to obtain the best results in different techniques and preparations we recommend determining optimal working dilution by titration test.

References

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