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# Product Information

**Anti-Cre antibody ,Mouse monoclonal**
clone 7-23, purified from hybridoma cell culture

Catalog Number **C7988**

## Product Description

Monoclonal Anti-Cre (mouse IgG1 isotype) is derived from the 7-23 hybridoma produced by the fusion of mouse myeloma cells (X63-Ag8.653) and splenocytes from BALB/c mice immunized with Cre protein. The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagent (Catalog Number ISO2). The antibody is purified from the culture supernatant of hybridoma cells grown in a bioreactor.

Monoclonal Anti-Cre recognizes Cre recombinase protein (~38 kDa). The product is useful in ELISA,[1] immunoblotting,[1] immunoprecipitation, immuno-histochemistry,[2] immunocytochemistry,[1] and flow cytometry.[1]

Cre recombinase belongs to the integrase family of proteins that are site-specific recombinases. The protein has a molecular mass of 38 kDa and is expressed in P1 bacteriophage. The enzyme is capable of performing DNA recombination between two recognition sites called *loxP*.[3] Each one of these sites (34 bp long) contains a two 13 bp palindromic flanking sequences and in between them is a core spacer sequence 8 bp long. In the recombination process each palindromic half of each *loxP* site binds to one Cre molecule and thus when two *loxP* sites are brought together during recombination, a tetramer of Cre recombinase is formed.[3] The recombination event takes place within the spacer area of the two *loxP* sites.

Two major features make the Cre/*loxP* system a useful tool for gene manipulation in mammalian systems. The first is the fact that the Cre enzyme does not need any cofactor for its activity, and thus its activity is dependent mainly on its expression. The second advantage is that the probability for the occurrence of a *loxP* site in a mammalian genome is very rare.[3]

Therefore, the Cre/*loxP* system can be used for mutagenesis in mammalian systems by producing deletions, gene replacements, insertions (knockin), conditional gene targeting (knockout), and point mutations.[3-4] This is achieved by inserting the *loxP* sites flanking the sequence of interest and applying the Cre enzyme on this construct, either *in vitro* or *in vivo*.

Monoclonal antibodies to the Cre protein are an important tool for the identification of Cre recombinase in different mammalian systems.

## Reagents

The product is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative

Antibody Concentration: ~2 mg/mL.

## Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses.

## Storage/Stability

For continuous use, store at 2–8 °C for up to one month.

For extended storage, the solution should be frozen 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. Working dilution samples should be discarded if not used within 12 hours.

**Procedure**
The whole procedure should be performed at room temperature.

1. Separate proteins from sample lysates using a standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protocol. Load 2.5–20 μg total lysate protein per lane. The amount of extract loaded 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 5% non-fat dry milk in phosphate buffered saline (PBS, Catalog Number D8537) for 60 minutes.

4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20 (Catalog Number P3563).

5. Incubate the membrane with anti-Cre antibody as the primary antibody in PBS containing 1% BSA, 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 Goat anti-mouse Fab, Peroxidase conjugate (Catalog Number A2304) 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.

**Product Profile**
A working dilution of 0.5-1 μg/mL is determined by immunoblotting using recombinant Cre recombinase.

Note: In order to obtain best results in various techniques and preparations, it is recommended to determine optimal working dilutions by titration.

**References**
1. Schwenk, F., et al., J. Immunol. Meth., **207**, 203-212 (1997).
2. Tsien, J. Z., et al., Cell, **87**, 1317-1326 (1996).
3. Nagy, A., Genesis, **26**, 99-109 (2000).
4. Rajewsky, K., et al., J. Clin. Invest., **98**, 600-603 (1996).



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