



Mouse anti-CTLA4

Cat. No.: MSK116-05 (0.5 ml concentrate); MSG116 (6 ml ready-to-use).

Instructions for use

Intended use

The antibody is used to localize CTLA4 protein in tissue sections of formalin-fixed, paraffin-embedded tissue. For use as an in vitro diagnostic.

Specifications

Specificity:	Human CTLA4
Clone:	UMAB249
Isotype:	Mouse IgG1
Immunogen:	Recombinant protein (<i>full lenght</i>) corresponding to human CTLA4.
Species reactivity:	human +, others not tested

Summary and Description

CTLA4 (*Cytotoxic T-lymphocyte associated protein 4*, CD152) is a negative regulator of the T cell-mediated immune response.

The negative regulator CTLA4 and the stimulatory regulator CD28 are receptors for the common ligands CD80 and CD86, whereby CTLA4 has a higher binding affinity for these and can thus displace CD28. Ultimately, CTLA4 thus specifically downregulates the activity of CD4-positive T helper cells.

Immunohistochemical detection of the CTLA4 protein is of great importance for the use of several new cancer therapeutics from the group of immune checkpoint inhibitors.

Reagent provided

Mouse monoclonal antibody in buffer solution with protective protein and preservative for stabilization in the formats:

Concentrate: 0.5 ml (Cat.No. MSK116-05)

Ready-to-use: 6 ml (Cat.No. MSG116)

Dilution of primary antibody

Zytomed Systems concentrated antibody must be diluted depending on the detection system used. The final working dilution must always be determined by the user. If other detection systems or protocols are used, validation by an experienced specialist is necessary. Recommendations from Zytomed Systems can be found in the section "Staining protocol".

Storage and handling

The antibody should be stored at 2-8°C without further dilution. If dilutions of the antibody are necessary, a suitable dilution buffer (e.g. article no. ZUC025 from Zytomed Systems) should be used for this purpose. The diluted antibody can be stored at 2-8°C after use. The stability of this prepared working solution depends on various factors and must be verified by appropriate controls.

The supplied antibody is usable until the expiration date indicated on the label when stored at 2-8°C. The antibody must not be used beyond the expiry date. Positive and negative controls must be carried in parallel with the test material. If unexpected staining or deviations from expected staining results are observed that are presumably due to the antibody, please contact the manufacturer.

Precautions

Application by trained personnel.

Safety data sheets for technical personnel are available on request.

Wear protective equipment to avoid eye, skin or mucous membrane contact with the reagent. If you come into contact with the reagent in a sensitive area, wash it with plenty of water. Microbial contamination of the reagents should be avoided, otherwise non-specific staining may occur.

The sodium azide (NaN_3) used for stabilization is not considered a hazardous substance in the present concentration. Sodium azide accumulations can lead to the formation of highly explosive metal azides in drain pipes made of lead and copper. To avoid such azide accumulations in drain pipes, flush with plenty of water after disposal. The safety data sheet for the pure substance is available on request.

Staining procedure

Follow the recommendations for the antibody below. Please also consider the information for the staining protocol in the package insert of the detection system you are using.

Parameters

*Pretreatment
*Control fabric
*Use dilution
*Incubation time

Zytomed Systems Recommendations

HIER = Thermal antigen unmasking (HIER) in citrate buffer pH 6.0
Tonsil
1:100 (for concentrates)
60 minutes

Quality control

The recommended control tissue for this examination is tonsil. We recommend performing a positive and a negative control for each staining run. Also consider the package insert of the detection system you are using for general quality control measures.

Troubleshooting

If an unusual coloration occurs, please check the package insert of the detection system for any indications or contact the manufacturer.

Expected results

The antibody shows a positive staining result in formalin-fixed, paraffin-embedded tissue in the cytoplasm and cytoplasmic membrane of T lymphocytes. For more details on the expression pattern of CTLA4, please refer to the "Summary and Explanation" section. Interpretation of staining results is the responsibility of the user. Each experiment should be verified by a medically established method or diagnostic product.

Limitations of the Procedure

Immunohistochemistry is a complex method combining histological as well as immunological detection methods. Tissue processing or sample handling prior to actual immunohistochemistry can lead to inaccurate results if guidelines are not followed (Nadji and Morales, 1983). Endogenous peroxidase activity, pseudoperoxidase activity in erythrocytes, or endogenous biotin content may cause nonspecific staining depending on the detection system used. Tissue containing hepatitis B surface antigen (HBsAg) may cause false positive results when detection systems containing HRP (horse radish peroxidase) are used (Omata *et al.*, 1980). Inadequate counterstaining or incorrect blanketing may affect the interpretation of results.

Zytomed Systems guarantees that the product meets all described requirements if stored and handled correctly until the expiration date. No further guarantees can be given.

Performance characteristics

Zytomed Systems has performed studies regarding the performance of the antibody in combination with a standard detection system. The product was found to be sensitive and specific with respect to the antigen.

Bibliography

Buchbinder EI, McDermott DF. Clin Therap 37:755-763, 2015. Shin DS, Ribas A. Curr Opin Immunol 33:23-35, 2015.
Wing K *et al.* Science 322:271-275, 2008. Baecher-Allan C *et al* Semin Immunol 16:89-98, 2004.
Azuma M *et al.* Nature 366:76-79, 1993. Hathcock KS *et al.* Science 262:905-907, 1993
Schwartz RH. Cell 71:1065-1068, 1992. Nadji M, Morales AR. Ann N Y Acad Sci 420:134-138, 1983.
Omata M *et al* Am J Clin Pathol 73:626-632, 1980.



www.zytomed-systems.de

Zytomed Systems GmbH •
Anhaltinerstraße 16 • 14163 Berlin,
Germany • Tel: (+49) 30-804 984 990

Explanations of the symbols on the product label

Symbols are used in accordance with ISO 15223-1. Further symbols on the product label might be:



GSH07: Warning / Attention

RUO

For Research Use Only