CONFIRM anti-CD8 (SP57) Rabbit Monoclonal Primary Antibody

REF 790-4460

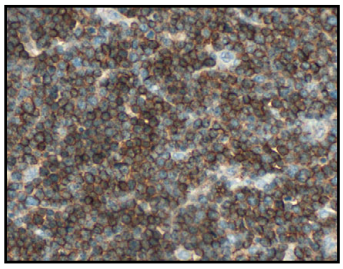

Figure 1. CONFIRM anti-CD8 (SP57) membrane staining of T cells in lymphoma.

INTENDED USE

Ventana Medical Systems' (Ventana) CONFIRM anti-CD8 (SP57) Rabbit Monoclonal Primary Antibody (CONFIRM anti-CD8 (SP57)) is intended for the qualitative detection of CD8 in sections of normal and neoplastic human tissues. The CD8 glycoprotein is present on cytotoxic/suppressor T lymphocytes that recognize antigen in the context of MHC class 1 molecules. CD8 positive staining results may aid in identifying T-cell lymphomas and in identifying the T cytotoxic/suppressor cell subset of T lymphocytes in normal tissues. The antibody is intended for qualitative staining in sections of formalin-fixed, paraffin-embedded tissue.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information and proper controls.

This antibody is intended for *in vitro* diagnostic (IVD) use.

SUMMARY AND EXPLANATION

CD8 is a heterodimeric, disulphide linked, transmembrane glycoprotein found on the cytotoxic-suppressor T cell subset representing 30-35% of peripheral blood T cell lymphocytes,[1,2] but not on B cell lymphocytes. CD8 is also present on 80% of thymocytes,[2] at a lower level on approximately 30-50% of natural killer cells,[3] and in a subpopulation of bone marrow cells. CD8 acts as a coreceptor for the T cell receptor and interacts directly with MHC class I molecules during T cell activation.[4,5] Anti-CD8 can be used as part of a panel of antibodies to classify T cell disorders, including T cell lymphomas, and to differentiate between helper and cytotoxic T cells.[6,7]

REAGENT PROVIDED

CONFIRM anti-CD8 (SP57) contains sufficient reagent for 50 tests.

One 5 mL dispenser of CONFIRM anti-CD8 (SP57) contains approximately 1.75 µg of a rabbit monoclonal SP57 antibody.

The antibody is diluted in 0.05 M Tris-HCL with 1% carrier protein and a preservative.

Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 0.35 µg/mL.

CONFIRM anti-CD8 (SP57) is a Protein A purified recombinant rabbit monoclonal antibody.

Refer to the appropriate Ventana detection kit package insert for detailed descriptions of: (1) Principles and Procedures, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents such as Ventana detection kits (i.e., *ultra*View Universal DAB Detection Kit), and ancillary components, including negative and positive tissue control slides, are not provided. Antibody Diluent (REF 251-018) is recommended for use at the ultraBlock step.

STORAGE

Store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with Ventana detection kits and a Ventana automated slide stainer. The recommended tissue fixative is 10% neutral buffered formalin.[8] Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. This product contains 1% or less bovine serum which is used in the manufacture of the antibody.
3. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
4. Avoid microbial contamination of reagents.
5. Consult local and/or state authorities with regard to recommended method of disposal.

STAINING PROCEDURE

Ventana primary antibodies have been developed for use on a Ventana automated slide stainer in combination with Ventana detection kits and accessories. A recommended staining protocol for the BenchMark XT and BenchMark ULTRA instruments with *ultra*View Universal DAB Detection Kit is listed in Table 1.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument's Operator's Manual. Refer to the appropriate Ventana detection kit package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocol for CONFIRM anti-CD8 (SP57) with *ultra*View Universal DAB Detection Kit. Antibody Diluent (REF 251-018) is recommended for use at the ultraBlock step on BenchMark XT/BenchMark ULTRA instruments.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Standard Cell Conditioning 1
Enzyme (Protease)	None required
Antibody (Primary)	BenchMark XT instrument Approximately 16 minutes, 37°C BenchMark ULTRA instrument Approximately 20 minutes, 36°C
ultraBlock (Recommended)	8 Minutes
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing Reagent, 4 minutes

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".[9]

POSITIVE TISSUE CONTROL

Examples of positive control tissues for this antibody are normal tonsil and T cell lymphoma.

STAINING INTERPRETATION

The staining pattern for CONFIRM anti-CD8 (SP57) is cell membrane.

SPECIFIC LIMITATIONS

This antibody has been optimized for a 16 minute incubation time on a BenchMark XT and 20 minute incubation on the BenchMark ULTRA instrument in combination with *ultra*View Universal DAB Detection Kit (REF 760-500). The user must validate results obtained with this reagent. Non-specific staining of some normal epithelia, as well as adenocarcinomas and carcinomas, were noted with this antibody. The use of an optional blocking reagent (Antibody Diluent REF 251-018) eliminated this non-specific staining without compromising the specific reactivity and is therefore recommended. Normal epithelia where non-specific staining was noted without ultraBlock include colon and prostate, and for neoplasms, ovarian serous papillary adenocarcinoma, breast lobular carcinoma and esophageal squamous cell carcinoma.

PERFORMANCE CHARACTERISTICS

1. Specificity of CONFIRM anti-CD8 (SP57) was determined by testing formalin-fixed, paraffin-embedded normal and neoplastic tissues.

   For normal tissues, results are as follows: (0/3) cerebrum, (0/3) cerebellum, (0/3) adrenal gland, (0/3) ovary, (0/3) pancreas, (0/3) parathyroid gland, (0/3) hypophysis, (0/3) testis, (0/3) thyroid gland, (0/3) breast, (3/3) spleen, (3/3) tonsil, (3/3) thymus, (3/3) bone marrow, (0/3) lung, (0/3) heart, (0/3) esophagus, (0/3) stomach, (0/3) intestine, (0/3) colon, (0/3) liver, (0/3) salivary gland, (0/3) kidney, (0/3) prostate, (0/3) uterus, (0/3) cervix, (0/3) striated muscle, (0/3) skin, (0/3) nerve, (0/3) mesothelium and lung.

2. Sensitivity of CONFIRM anti-CD8 (SP57) was determined by testing a variety of formalin-fixed, paraffin-embedded target specific neoplastic tissues.

   For neoplastic tissues, results are as follows:(0/1) glioblastoma, (0/1) atypical meningioma, (0/1) malignant ependymoma, (0/1) malignant oligodendroglioma, (0/1) ovarian serous papillary adenocarcinoma, (0/1) ovarian mucinous papillary adenocarcinoma, (0/1) islet cell carcinoma, (0/1) pancreatic adenocarcinoma, (0/1) seminoma, (0/1) embryonal carcinoma, (0/1) medullary carcinoma, (0/1) papillary carcinoma, (0/1) intraductal carcinoma, (0/1) breast lobular carcinoma in situ, (0/1) invasive ductal carcinoma, (0/1) diffuse B cell lymphoma, (0/1) small cell undifferentiated carcinoma, (0/1) lung squamous cell carcinoma with necrosis, (0/1) lung adenocarcinoma, (0/1) esophageal squamous cell carcinoma, (0/1) esophageal adenocarcinoma, (0/1) mucinous adenocarcinoma, (0/1) small intestine adenocarcinoma, (0/1) small intestine intermediate grade interstitialoma, (0/1) colon adenocarcinoma, (0/1) colon intermediate grade interstitialoma, (0/1) rectum adenocarcinoma, (0/1) rectum intermediate grade interstitialoma, (0/1) hepatocellular carcinoma, (0/1) heptaoblastoma, (0/1) clear cell carcinoma, (0/1) prostate adenocarcinoma, (0/2) transitional cell carcinoma, (0/1) leiomyoma, (0/1) endometrial adenocarcinoma, (0/1) endometrial clear cell carcinoma, (0/2) uterine cervix squamous cell carcinoma, (0/1) embryonal rhabdomyosarcoma, (0/1) malignant melanoma, (0/1) basal cell carcinoma, (0/1) squamous cell carcinoma, (0/1) neurofibroma, (0/1) neuroblastoma, (0/1) epithelial malignant mesothelioma, (1/3) diffuse malignant lymphoma, (0/1) low grade leiomyosarcoma, (0/1) osteosarcoma, (0/1) spindle cell rhabdomyosarcoma, (0/1) intermediate grade leiomyosarcoma.

   (1/96) B cell lymphoma (see note below), (0/4) small lymphocytic lymphoma, (0/3) follicular lymphoma, (0/13) Hodgkin's lymphoma, (0/5) anaplastic large cell lymphoma, (0/6) mantle cell lymphoma, (0/11) mucosa associated lymphoma, (5/10) T cell lymphoma, (0/1) Burkitt like lymphoma.

   Note: The single B cell lymphoma that exhibited weak CD8 positivity was also demonstrated to be CD20 positive, CD3 negative. While rare, such lineage infidelity in tumors is reported in the literature.[10]

3. Inter-lot reproducibility was determined by testing 3 lots across 3 tissue types on a BenchMark XT instrument. 18 out of 18 tested across all 3 lots scored equivalently.
4. Inter-run repeatability was determined by staining 2 multi-tissue blocks (3 tissues per block for a total of 6 tissues) across 5 slides on a BenchMark XT instrument over a five day non-consecutive period. 150 out of 150 samples tested scored equivalently.
5. Intra-run repeatability was determined by staining 2 multi-tissue blocks (3 tissues per block for a total of 6 tissues) across 14 slides on a BenchMark XT instrument. 84 out of 84 samples tested scored equivalently.
6. Intra-platform repeatability was determined by staining 2 multi-tissue blocks (3 tissues per block) across 5 slides on 3 BenchMark XT instruments. 89 out of 90 samples tested scored equivalently.
7. Intra-platform repeatability was determined by staining 1 multi-tissue blocks (3 tissues per block) across 5 slides on 3 BenchMark ULTRA instruments. 45 out of 45 samples tested scored equivalently.
8. Inter-platform repeatability was determined by staining 1 multi-tissue block (3 tissues per block) across 5 slides on 3 BenchMark XT instruments and 3 BenchMark ULTRA instruments. 89 out of 90 samples tested scored equivalently.
9. Compatible with BenchMark XT and BenchMark ULTRA instruments and with *i*VIEW DAB with Endogenous Biotin Blocking Kit (REF 760-050) and *ultra*View Universal DAB Detection Kit with a blocking reagent (REF 251-018).

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