

Perforin (MRQ-23)

For In Vitro Diagnostic Use (IVD)

English: Instructions For Use

Presentation

Anti-Perforin is a mouse monoclonal antibody from supernatant diluted in phosphate buffered saline, pH 7.4, with protein base, and preserved with sodium azide.

Applications

Perforin is a pore-forming protein that leads to osmotic lysis of the target cells and subsequently enables granzymes to enter the target cells and activate apoptosis, the cell death program. The expression of perforin is upregulated in activated CD4+ T cells, but in NK cells the expression is constitutively very high and stable. Perforin expression can also be stimulated in some activated CD4+ T cells. CD8+ T cells and NK cells use both the perforin/granzyme and the Fas/FasL pathways, while CD4+ T cells preferentially use the Fas/FasL pathway.

Although some investigators report a cytolytic potential of CD4+ T cells, it appears more likely that CD8+ T cells are the major effector population in Th1- associated inflammatory skin diseases. The role of perforin-mediated cytotoxicity has been demonstrated in various autoimmune diseases. In vitro and in vivo studies suggest that the cytotoxicity of CTLs may be mediated by cytotoxic granules in certain inflammatory diseases in humans. In addition, it seems that T-cell cytotoxicity against keratinocytes is mediated by perforin in some inflammatory skin diseases.

Other authors suggest that perforin may have a dual role in alloimmune response (organ transplant applications). In one regard, it has a cytolytic function in acute rejection, and, in contrast, it may be responsible for downregulating both CD4- and CD8-mediated alloimmune response.

Reactivity	Paraffin, frozen
Control	Spleen
Visualization	Perinuclear cytoplasmic
Stability	Up to 36 months; store at 2-8°C
Isotype	IgG ₁

Antibody color does not affect performance

Description	Ventana®* Cat. No.
50 test dispenser	760-4394

Preparation

1. Cut 3-4 µm section of formalin-fixed, paraffin-embedded tissue and place on positively charged slides; dry overnight at 58° C.

Recommended Ventana®* Staining Procedure (UltraView™)

1. Load slides, antibody, and UltraView™ detection kit dispensers onto BenchMark®* instrument.
2. Select CC1 Mild pretreatment.
3. Antibody incubation should be set for 16 minutes at 37° C.
4. Start the run.
5. When the staining run is complete, move slides from instrument and rinse well with wash buffer.
6. Coverslip.

Recommended Ventana®* Staining Procedure (iView™)

1. Load slides, antibody, and iView™ detection kit dispensers onto BenchMark®* instrument.
2. Select CC1 Standard pretreatment.
3. Antibody incubation should be set for 32 minutes at 37° C.
4. Start the run.
5. When the staining run is complete, move slides from instrument and rinse well with wash buffer.
6. Coverslip.

References

1. Chu PG, Chang KL, Arber DA, Weiss LM. Practical applications of immunohistochemistry in hematolymphoid neoplasms. *Ann Diagn Pathol.* 1999 Apr;3(2):104-33. Review.
2. Bittmann I, Müller C, Behr J, Groetzner J, Frey L, Löhrs U. Fas/FasL and perforin/granzyme pathway in acute rejection and diffuse alveolar damage after allogeneic lung transplantation-a human biopsy study. *Virchows Arch.* 2004 Oct;445(4):375-81. Epub 2004 Jul 29.
3. d'Amore ES, Menin A, Bonoldi E, Bevilacqua P, Cazzavillan S, Donofrio V, Gambini C, Forni M, Gentile A, Magro G, Boldrini R, Pillon M, Rosolen A, Alaggio R. Anaplastic large cell lymphomas: a study of 75 pediatric patients. *Pediatr Dev Pathol.* 2007 May-Jun;10(3):181-91.

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Inline Dispenser Preparation, Handling & Storage Instructions

Preparing For Use:

Where Used: For NexES® IHC, BenchMark® Series and Discovery® automated instruments, software version 8.0 and higher.

STEP 1: Shipping Key Removal

To remove the Shipping Key (shown in Figure A), remove the Nozzle Cap, hold the dispenser upright and pull the Key Tab to disengage it from each end. DO NOT cover the nozzle tip as it could permanently damage the dispenser. DO NOT depress the dispenser while removing the key as it could waste reagent. Discard the shipping key.

STEP 2: Preparing the Dispenser for Use

Remove the Nozzle Cap and place on the Nozzle Cap Holder. Fluid may be present inside the Nozzle Cap. Install the dispenser on the reagent carousel. The Inline Dispenser has been designed to be "Prepared for Use" by the NexES software Version 8.0 or higher. Before each run, the software will detect a new dispenser on the carousel and prime it automatically. Manually priming the dispenser is not necessary and should NEVER be done as it could waste reagent and decrease the number of available dispenses.

Note - All earlier software installations: After removing the shipping key, remove the nozzle cap and CHARGE THE DISPENSER BY RAPIDLY PUMPING 3 to 4 TIMES, keeping the dispenser in an upright position. Charging is only necessary prior to first time use. (See Inspect Prime Before Use section.)

STEP 3: Dispenser Storage & Handling

To insure reliable operation, the dispenser must always be capped when not in use and should NEVER be manually dispensed. (See the Do's and Don't section.)

Do's and Don't-Do:

1. Check priming chamber and meniscus before each use. (See Inspect Prime Before Use).
2. Store nozzle cap on dispenser. A holder is provided.
3. Cap dispenser when not in use to prevent evaporation. Dispensers mounted on the reagent tray can be capped (from underneath the tray) when not in use.
4. Store dispensers in an upright position in a rack and on the reagent carousel.
5. When mounting the dispenser on the carousel, grasp the coupler to avoid accidental manual dispensing.

DON'T:

1. Do not manually dispense when inverted (upside down). Prime will be lost and may be impossible to restore.
2. Do not manually dispense with the nozzle cap in place. This can permanently damage the dispenser.
3. Do not manually dispense or prime prior to each use. This is not necessary and wastes reagent.
4. Do not hold the barrel in the down position. Fluid can leak from the dispenser when the barrel is depressed.
5. Do not stack carousels with dispensers installed. This can cause the dispensers to leak.

Inspect Prime Before Use:

Remove the nozzle cap and refer to Fig. B.

Dispenser Is Ready For Use When:

1. A meniscus is present in the area shown in Figure B.
2. The priming chamber contains liquid.

If one or both of these conditions is not satisfied, consult Signs of Trouble and What to Do section.

Signs Of Trouble & What To Do:

1. Priming chamber empty. If there is no liquid in the priming chamber, re-prime the dispenser (see Re-Priming the Dispenser section).
2. Meniscus absent. If no meniscus is visible in the nozzle area, manually charge the dispenser once. If this does not resolve the condition, re-prime the dispenser (see Re-Priming the Dispenser section). If condition reoccurs, contact your local Ventana Customer Support Center.
3. Leaking dispenser. External fibers (from clothing or other sources) can cause dispenser to leak. Use in a clean environment.
4. Blocked dispenser. The normal performance characteristics of the dispenser are such that particulates (i.e., fibers, precipitation) could cause a dispenser blockage. A sign of blockage could include higher reagent volume than expected, remaining within the dispenser, after a period of use. Blockage is also evidenced by the failure of the dispenser to yield fluid upon manual dispense, which can be tested by the steps listed in the Re-Priming the Dispenser section. If blockage is suspected (or if foreign material is observed in the dispenser), contact the Ventana Customer Support Center.

NOTE: DO NOT manually dispense or prime the dispenser unless absolutely necessary. Although Ventana pre-filled dispensers have been overfilled to insure a sufficient number of tests, manual dispensing or priming can cause insufficient tests remaining in the dispenser and may cause undesirable staining results.

Consult individual reagent package inserts for information on the utilization of appropriate Quality Control Procedures.

Re-priming The Dispenser:

Once primed, the dispenser should not lose prime if handled correctly. If re-priming is necessary, proceed as follows:

1. Aim the dispenser tip at a waste container. Remove the nozzle cap and depress the barrel (top of the dispenser). This should dispense a drop.
2. If no drop is dispensed, repeat Step 1, above, several times until a drop is ejected.
3. If a drop is ejected, proceed with instructions in Inspect Prime Before Use on this page.
4. If no drop is ejected, or inspection for prime (Step 3) fails, contact your local Ventana Customer Support Center.

Contacting Ventana Technical Consultation Center

If your dispenser does not look or perform as expected, please contact your local Ventana Customer Support Center for advice or return information. Please have the dispenser Lot Number (from the reagent label) handy when you call.

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