CELL MARQUE 6600 Sierra College Blvd. • Rocklin, CA 95677 USA • 800-665-7284

Rev. 2.1
KEY-CODE CMC44480021

PD-1 (MRQ-22)

(also known as NAT)

For In Vitro Diagnostic Use (IVD)
English: Instructions For Use

Presentation
Anti-PD-1 is a mouse monoclonal antibody from ascites diluted in phosphate buffered saline, pH 7.4, with protein base, and preserved with sodium azide.

Applications
Programmed death-1 (PD-1) is a member of the CD28 family of receptors that includes CD28, cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), inducible costimulator (ICOS), and B- and T-lymphocyte attenuator. These receptors play a role in the cellular immune response. For example, CD28 serves as a costimulatory receptor that enhances T-cell activation, whereas CTLA-4 serves as an inhibitor of T-cell activation. PD-1 also has an inhibitory function on T cells and B cells, and is important in peripheral tolerance. There are at least 2 ligands for PD-1, PD-L1, and PD-L2, which are expressed on a range of cells.

CD28 is constitutively expressed on most or all CD4+ T cells and approximately 50% of CD8+ T cells, whereas CTLA-4 is not expressed on resting T cells. PD-1 is also expressed on activated T cells, B cells, and myeloid cells. Iwai and coworkers studied the microanatomic distribution of PD-1 in human tonsil and found that PD-1 is expressed on most T cells and a small subset of B cells in the light zone of germinal centers, but not elsewhere in the tonsil. On that basis, it was postulated that PD-1 may play a role in the process of clonal selection of centrocytes, which occurs in this subanatomic site in germinal centers.

PD-1 is a new marker of angioimmunoblastic lymphoma and suggests a unique cell of origin for this neoplasm. Unlike CD10 and bcl-6, PD-1 is expressed by few B cells, so it may be a more specific and useful diagnostic marker in angioimmunoblastic lymphoma. It also seems to stain a greater percentage of CD3-positive neoplastic cells in angioimmunoblastic lymphoma than either CD10 or bcl-6. In addition, PD-1 expression provides new evidence that angioimmunoblastic lymphoma is a neoplasm derived from germinal center-associated T cells. PD-1 expression in angioimmunoblastic lymphoma lends further support to this model of T-cell oncogenesis, in which specific subtypes of T cells may undergo neoplastic transformation and result in specific distinct histologic, immunophenotypic, and clinical subtypes of T-cell neoplasia. Chtanova and coworkers identified a number of genes that are specifically up-regulated in expression in germinal center-associated T cells, in addition to PD-1. It will be interesting to determine whether the expression of these other genes can be studied by immunostaining in angioimmunoblastic lymphoma and other lymphoid neoplasms. Furthermore, it may be possible that one or more of these new markers of angioimmunoblastic lymphoma, such as PD-1, may provide the basis for an immunotherapeutic approach to the treatment of angioimmunoblastic lymphoma, similar to the use of anti-CD20 and anti-CD52 immunotherapy in B-cell neoplasia.

Reactivity	Paraffin, frozen
Control	Lymph node, tonsil
Visualization	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	760-4448

EC REP EMERGO EUROPE
Molenstraat 15, 2513 BH, The Hague, NL.

CE *Material Safety Data Sheet available upon request.*

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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

1. Load slides, antibody, and UltraView™ 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

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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.

CONTACT INFORMATION:
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EC REP

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Figure A

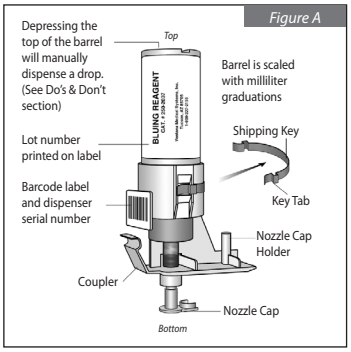

Figure B