

p21^{WAF1} (DCS-60.2)

For In Vitro Diagnostic Use (IVD)

English: Instructions For Use

Presentation

Anti-p21^{WAF1} is a mouse monoclonal antibody from ascites diluted in phosphate buffered saline, pH 7.4, with protein base, and preserved with sodium azide.

Applications

p21 is a nuclear 21kD protein, a product of WAF1/CIP1 gene. It is a cyclin-dependent kinase inhibitor 1A (p21, Cip1), also known as CDKN1A, which in humans is encoded by the CDKN1A gene located on chromosome (6p21.2). It is a constituent of a large complex of nuclear proteins, including cyclins, cyclin dependent kinases, and PCNA. Cell cycle progression is regulated by cyclins and their cognate Cdks. p21 inhibits the activity of each member of the cyclin/Cdk family. The expression of this gene acts as an inhibitor of the cell cycle during G1 phase and is tightly controlled by the tumor suppressor protein p53. Normal cells generally display a rather intense nuclear p21 expression. Loss of p21 expression has been associated with poor prognosis in several carcinomas (gastric carcinoma, non-small cell lung carcinoma, thyroid carcinoma).

Reactivity	Paraffin, frozen
Control	Colon carcinoma, colon, tonsil
Visualization	Cytoplasmic, nuclear
Stability	Up to 36 months; store at 2-8°C
Isotype	IgG _{2a}

Antibody color does not affect performance

Description	Ventana[®] Cat. No.
50 test dispenser	760-4453

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 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.

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. DiGiuseppe JA, Redston MS, et al. p53-independent expression of cyclin-dependent kinase inhibitor p21 in pancreatic carcinoma. *Am J Pathol* 1995;147:884-8.
2. Yoshitito Gomyo, Mitsuyuki Ikeda, et al. Expression of p21(waf1/cip1/sdi1), but not p53 protein, is a factor in the survival of patients with advanced gastric carcinoma. *Cancer* 1997; 79: 2067-2072.
3. Ikeguchi M, Saito H, et al. Expression of p53 and p21 are independent prognostic factors in patients with serosal invasion by gastric carcinoma. *Dig Dis Sci* 1998;43:964-70
4. Marone M, Bonanno D, et al. Survival and cell cycle control in early hematopoiesis: role of bcl-2 and the cyclin dependent kinase inhibitors p27 and p21. *Leuk Lymphoma* 2003 43(1):51-7.
5. Kwon MS, Lee YI, et al. p21 as a prognostic factor in non-small cell lung carcinomas. *Pathol Res Pract*. 2006;202(12):849-56
6. Elettra Merola, Eliseo Mattioli, et al. Immunohistochemical evaluation of pRb2/p130, VEGF, EZH2, p53, p16, p21waf-1, p27, and PCNA in Barrett's Esophagus. *J Cell. Physiol.* 2006 207:512-519.

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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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