

Trilogy™ (Concentrate)

For In Vitro Diagnostic Use (IVD)
Instructions for use

Presentation

Trilogy™, developed and manufactured by Cell Marque™ Corporation, is a product that combines the three pretreatment steps: deparaffinization, rehydration, and unmasking in immunohistochemistry stains. The product is completely biodegradable and non-toxic.

Availability	Catalog No.	Dilution	Volume
	920P-04	Concentrated	50 ml of 20X
	920P-06	Concentrated	200 ml of 20X

prediluted concentrate

For working solution, dilute concentrated Trilogy™ with DI water 1:20. You also may dilute the entire 200 ml of reagent to make up to 4 liters of working solution of Trilogy™. (Sample size Trilogy™ Concentrate is 50 ml and can be diluted with DI water to make up to 1 liter.)

Stability: Up to 36 months; store at 20-26°C

Applications

Trilogy™ may be used whenever immunohistochemistry staining is performed on formalin fixed, paraffin embedded tissue sections. Using this product encourages standardization of the pretreatment procedure, thereby producing more consistent, more reliable results.

One-Step Procedure

- Cut 3-4 micron sections and place on positively charged slides. Air Dry.
- Dry sections in incubator for at least 2 hrs. at 58° C.
- Place up to 24 slides in plastic slide rack*
- Place plastic slide rack into staining dish* and fill with approx. 200 ml of Trilogy™. Prepare a second staining dish filled with approx. 200 ml of Trilogy™ (This will be used as a hot rinse following processing time in the first staining dish.) Make sure that the staining dishes do not press against the pressure pin when viewed from the underside of the lid. Should this occur, there will be interference with the locking mechanism and make it difficult to unlock and open the lid following completion of the pressure cycle.
- Either of the following two heating methods may be used; however, the pressure cooker method often produces superior results:
 - Electric Pressure Cooker (preferred method), follow steps 6-12.
 - Conventional Steamer Method: place both staining dishes (one of which contains the slide rack) in a steamer, cover and steam for 30 minutes. Skip to step 13.
- Elec. Pressure Cooker Method: Place approx. 700 ml of water in the base of unit and set inverted (i.e. legs pointing upward) rack into the water. Put both staining dishes inside the pressure cooker. Lock pressure cooker lid in place atop the pressure cooker and make sure vent switch located on the lid handle is in the closed position. Make sure the pressure weight is rotated until it is completely seated in its receptacle otherwise the pressure cooker will not be able to build pressure. Using the buttons on the control panel, set pressure mode for "high" and move "up" arrow until you reach 15 then press the "start" button.

- Timer will start to count down when the correct pressure and temperature are reached. After timer goes to "zero", push "off" button.
- Wait approximately 5 minutes then move the vent switch away from the closed position so as to release pressure.
- The red pin atop the lid will descend when all the pressure is released so you can safely remove the lid. Simply press the red button located on the base of the lid handle while rotating the lid to the open position.
- Transfer slides from first container to the hot rinse (second container) using a forceps to lower the slide rack very slowly into the hot rinse solution. Caution: Since the hot rinse solution is superheated, rapid immersion of slide rack may cause sudden boil out of the hot solution.
- Agitate slides and let sit for 5 minutes (first solution can be discarded and you may reuse the hot rinse solution in the first container next time the procedure is performed.)
- Rinse slides in IHC Wash Buffer or deionized water and proceed with IHC protocol.
- Steamer Method: Immediately transfer slides from original Trilogy™ to fresh hot Trilogy™ in second staining dish (hot rinse), agitate and continue heating process for additional 30 minutes.
- Wash Trilogy™ off with IHC wash buffer or deionized water. The Trilogy™ contained in the staining dish into which slide rack was initially placed must now be discarded. The Trilogy™ in the second (hot rinse) staining dish may be reused as your initial Trilogy™ next time you repeat the process i.e. you may rotate the solutions once.
- Continue IHC staining according to procedure routinely employed.

Three-Step Procedure

- Cut and dry your paraffin sections as described above then deparaffinize and rehydrate your sections.
- Place plastic slide rack into staining dish* and fill with approx. 200 ml of Trilogy™
- Electric Pressure Cooker Method (preferred method): follow steps 6-12 above except use only one container of Trilogy™. After pressure cooker lid has been removed, allow slides and container to remain in place for 5 minutes then wash Trilogy™ off with IHC wash buffer or DI water.
 - Steamer Method: place one staining dish* into the steamer and steam for 60 minutes then wash Trilogy™ off with IHC wash buffer or deionized water.
- Continue IHC staining according to procedure routinely employed.

*Additional Items

Catalog No.	Description
976L	Electric Pressure Cooker (110V, 60Hz)
975L	Pair Plastic Staining Dishes & Slide Rack