

DEPARAFFINIZATION/ REHYDRATION AND ANTIGEN RETRIEVAL PROTOCOL FOR IHC

It is recommended that the Trilogy method (Cell Marque; www.cellmarque.com; Cat# CMX833-C) should be used for deparaffinization and antigen retrieval of HHV-6 antigens. This is the method used in our regular quality control of the slides. However, other deparaffinization and antigen retrieval methods will also work and have been tested.

One-Step Procedure

1. Cut 4-5 μm sections and place on slides. Air dry.
2. Dry sections in incubator for at least 2 hrs. at 58° C.
3. Place up to 24 slides in plastic slide rack.
4. Place plastic slide rack into staining dish and fill with approximately 200ml of Trilogy™. Prepare a second staining dish filled with approximately 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.
5. Either of the following two heating methods may be used; however, the pressure cooker method often produces superior results:
 - (i) **Electric Pressure Cooker** (preferred method), follow steps 6-12.
 - (ii) **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.
6. **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.
7. Timer will start to count down when the correct pressure and temperature are reached. After timer goes to "zero", push "off" button.
8. Wait approximately 5 minutes then move the vent switch away from the closed position to release pressure.
9. 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.
10. 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.
11. 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.)
12. Rinse slides in IHC Wash Buffer or deionized water and proceed with IHC protocol.
13. **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.
14. Wash Trilogy™ of 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.
15. Continue IHC staining according to procedure routinely employed.

IMMUNOHISTOCHEMISTRY (IHC)

1. Once slides are deparaffinized and the antigen is retrieved, cell lines and tissue sections can be analyzed by traditional IHC methods
2. Blocking and incubation conditions are performed with the normal procedure according to the appropriate antibody specifications.
3. If humidity is required, place the slide in the humidity chamber.

We recommend the use of DAKO's ENVISION+ System-HRP mouse (DAB) for immunostaining (DAKO Cat# K4006). This system requires 30 minutes of incubation with the primary antibody and 30 minutes incubation with the secondary antibody. This is the method used in our quality control of the antibodies for IHC.

For the DAKO's Envision system the Bioworld Consulting Laboratories' antibodies should be diluted 1/1,000

Other immunostaining systems can be used, but the dilution of the primary antibodies should be titrated out (between 1/500 and 1/1,000) for the best specific staining and lowest background staining.