

EB virus FISH Kit (20Test, Cat: EB1803)

For Research Use Only

Z-L-20180322-V3.0



Application :

This kit is used to detect EB virus in slides embedded with paraffin by FISH, including as following

- 3% Hydrogen peroxide (Solution 1)
- HCl buffer (Solution 2)
- Proteinase K buffer (Solution 3)
- EB Hybridization probe solution (Probe Solution 4)
- The second antibody(Antib-bio-HRP) (Solution 5)

Solutions and reagents :

- Xylol
- 100%、85%、70% ethanol
- DAB Substrate kit (DAB kit)
- Hematoxylin
- Neutral resin
- Distilled water
- PBS
- 2xSSC、0.1x SSC、0.5 xSSC

In Situ Hybridization :

All incubations were performed at room temperature unless otherwise indicated.

1. Put slides on the heating plate, heating under the temperature of 85°C for 15 min.
2. Prepare three cups of xylene solution. The heated slides were immersed in xylene solution , three times for five minutes ,and then added sequentially to 100, 85, and 75 percent ethanol for 3 min each time.
3. After washing with purified water, the slides were dipped in 3% hydrogen peroxide for 10-15min. After that, rinse with purified water for 2 minutes. **(Solution 1)**
4. Specimens were treated by covering with 0.2N HCl for 20min.Then, washed with PBS for 5min. **(Solution 2)**
5. Specimens were treated by covering the spots with Proteinase K for 15 min at 37°C.

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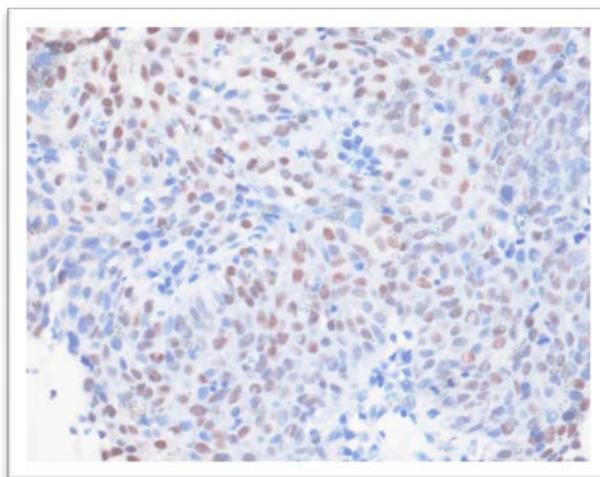
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- After that, washed by PBS for 5min , three times. **(Solution 3)**
- 50 μ L of this hybridization solution was placed on each slide and then cover with cover slips. Put them on the heating plate at 95°C for 5 min, followed by placing the slides at 37°C for 16h. Subsequently, the slides were placed in a solution of 2 x SSC to remove the slips. **(Probe Solution 4)**
 - Following consecutive washes in 1x and 0.1x SSC solution, cells were blocked using the second antibody. 50 μ L of this antibody solution was placed on each slide and incubated in a wet box for 1h. **(Solution 5)**
 - Slides were then washed in 2x SSC for 10min and 0.5x SSC for 5min.
 - Add 100 μ L of DAB (operated according to the product manual) to each specimen for 10min and washed by distilled water for 1min.
 - Add the hematoxylin to each specimen for 1min and then washed with PBS for 10min.
 - Put the specimen successively in 75%, 85% and 100% ethanol for 3min, and in xylol solution for 10min. Add with neutral resin and cover with cover glass. Air dry for 24h.
 - Afterwards, examine the slides under a microscope with proper filter set.

Detection: Signal on sections will present brown (DAB) or blue (NBT/BCIP) according to the labeled molecule.



- DAB Substrate kit (DAB kit) (Cat:DAB-EB002) 200.00RMB
 - Hematoxylin 50mL (Cat: EP-4500) 200RMB
 - Neutral resin 50mL (Cat: EP-4505) 120RMB
- Note: PBS and 2 x SSC are free, you can get them from our lab.

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