

IHC Protocol

BD PharMingen, Catalog number: 554149

Purified Mouse Anti-SV40 Large T antigen Antibody

DAKO, Cat No.: K681, LSAB kit (Peroxidase)

Updated on 4-15-02

1. Deparaffinized slides. (3 min each step Xylene-70% ETOH into 1X PBS)
2. Immerse slides in 600 ml 10mM Citric acid pH6.0. Microwave on (Power 9) for **37 min**. Cool down in room temperature for **30-60 min**
3. Quench endogenous Peroxidases **10 min** (1 part 30% H₂O₂ and 9 parts Methanol)

Wash 3X in 1X PBS pH 7.4, 3 min per changes.
4. Blocking @ Room Temperature **30 min**.
Blocking reagent from LSAB kit (Peroxidase)
5. 1° Ab @ Cold Room Overnight
(1:400) SV 40 Large T antigen antibody in 1X PBS pH 7.4 + 0.2% BSA
6. Wash in 1X PBS pH 7.4 @ RT **30 min** 5 mins per changes.
7. Secondary Ab @ Room Temperature, **60 min**
LINK: Biotinylated anti-rabbit and anti-mouse immunoglobulins from LSAB kit, Peroxidase.
8. Wash in 1X PBS, pH7.4 @ RT **30 min**, 5 mins changes.
9. Streptavidin from LSAB kit , @ Room Temperature for **30 mins**
10. DAB~5min. WEAR GLOVES
178 mL H₂O₂
20 ml 10X DAB buffer. Mix.
2 mL 5% DAB. Mix.
100 uL 30% H₂O₂. Mix.
Neutralize DAB with Bleach and dispose.
Wash **5 min** H₂O.

April 15, 2002

11. Counterstain in 0.1 % methylgreen, 3 min
Wash H₂O 3 times
12. 95% EtOH (3 second), 100% EtOH , I, II, III, Xylene I,II,III. 3 min
13. Coverslip.

10X PBS	1 L	4 L
Na₂HPO₄, anhydrous(M.W. 141.96)	11.5 g	46g
KH₂PO₄, (M.W. 136.09)	2 g	8g
KCl, (M.W. 74.56)	2 g	8g
NaCl , (M.W. 58.44)	80 g	320 g
Dilute to 1X before use and adjust pH to 7.4		

10X Citric acid, Trisodium Salt, Dihydrate. M.W. 294.10
Amresco, Cat No:0101

Dissolve 29.41 g into 1 L of H₂O
Dilute to 1X before use and adjust pH to 6.0 with glacial acetic acid

10X DAB Buffer
0.5 M Tris pH7.4
1 N Nacl

5% 3,3'-Diaminobenzidine, (DAB)
Amresco, Cat No. 0430-10g

10 g DAB in 200 ml dd H₂O
make 2 ml aliquote and store at -20C