

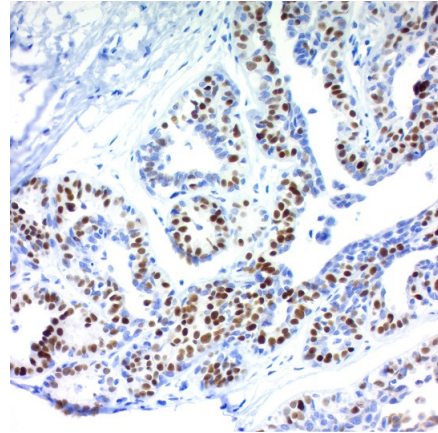
SensiTek HRP Anti-Polyvalent Staining System

Description: The SensiTek staining kit provides an unmatched combination of economy and sensitivity with incubation times of 20 minutes each for the Link Antibody and Enzyme Label.

Species of Origin: Goat
Antigen Specificity: Anti-Polyvalent (Mouse, Rat, Rabbit and Guinea Pig).
Preadsorbed Against: Human
Enzyme Conjugate: Horseradish Peroxidase
Chromogen Substrate: None Provided

Contains: 4x15ml Super Block.
 4x15ml SensiTek Anti-polyvalent.
 4x15ml SensiTek HRP.

Uses/Limitations: Not to be taken internally.
 For In-Vitro Diagnostic use.
 Histological applications.
 Do not use if reagents become cloudy.
 Do not use past expiration date.
 Use caution when handling reagents.
 Non-Sterile.



Control Tissue: Any FFPE tissue.


Ordering Information and Current Pricing at www.scytek.com


Storage: Store at 2-8°C.

Precautions: Avoid contact with skin and eyes.
 Harmful if swallowed.
 Follow all Federal, State, and local regulations regarding disposal.


Recommended, But Not Included:

| <u>Item #</u> | <u>Description</u> |
|---------------|---|
| PBE500 | Phosphate Buffered Saline + Tween 20 (10x) pH 7.4 |
| or TBE500 | Tris Buffered Saline + Tween 20 (10x) pH 7.5 |
| CPL500 | Citrate Plus |
| ADA500 | Peroxide Block for Image |
| ACT500 | DAB Chromogen/Substrate Kit (High Contrast) |
| HMM500 | Hematoxylin, Mayer's (Lillie's Modification) |
| BRT500 | Bluing Reagent |

Storage: 2° C  8° C

 ScyTek Laboratories, Inc.
 205 South 600 West
 Logan, UT 84321
 U.S.A.

 Emergo Europe
 Prinsessegracht 20
 2514 AP The Hague, The Netherlands


Procedure:

1. Rehydrate tissue slides.
2. In a glass or plastic (Autoclavable) Coplin jar, add 5 ml of Citrate Plus (CPL) and 45 ml of deionized water. *(Not included)*
3. Submerge slides in diluted Citrate Plus and loosely cap.
4. Add Distilled water to bottom of Autoclave or Pressure Cooker (about 1 inch deep in Pressure Cooker).
5. Place Coplin jar in Pressure Cooker or Autoclave.
6. Turn heat on and allow pressure to rise to 20-25 PSI.
7. Maintain pressure at 20-25 PSI for 5 minutes.
8. Turn off heat source and allow to cool.
9. When pressure has dropped to ambient, carefully remove lid or open door.
10. Using tongs, remove Coplin Jar and place on counter.
11. Once Coplin Jar cools to room temperature remove slides, rinse several times in buffer and proceed with staining as usual.
12. Apply Peroxide Block for Image Analysis (ADA) and incubate slide for 10-15 minutes. *(Not included)*
13. Rinse 3 times in buffer.
14. Apply Super Block, and incubate for 5 minutes at room temperature to block nonspecific background staining. **Note:** Do not exceed 10 minutes or there may be a reduction in desired stain.
15. Rinse 3 times in buffer.
16. Apply primary antibody and incubate according to manufacturer's protocol.
17. Rinse 3 times in buffer.
18. Apply SensiTek Anti-Polyvalent and incubate for 20 minutes at room temperature.
19. Rinse 3 times in buffer.
20. Apply SensiTek HRP and incubate for 20 minutes at room temperature.
21. Rinse 3 times in buffer followed by 1 rinse in DI water.

WARNING: DAB is a suspected carcinogen. Handle with care and dispose of according to all regulations.

22. Add 1 drop (40-50ul) DAB Chromogen (ACB) to each 1ml of DAB Substrate High Contrast (ACU), mix by swirling and apply to tissue for 5 minutes. *(Not included)*
23. Rinse 1 time in DI water.
24. Apply DAB Chromogen/Substrate mixture and incubate for a second 5 minute period.
25. Rinse 3 times in buffer.
26. Apply Hematoxylin, Mayer's (HMM) and incubate for 1 minute. *(Not included)*
27. Rinse 3 times in distilled water.
28. Apply Bluing Reagent (BRT) and incubate for 5-10 seconds. *(Not included)*
29. Rinse immediately in distilled or deionized water.
30. Dehydrate slides and clear in xylene or xylene substitute.
31. Coverslip using a permanent mounting media. *(Not included)*

Storage: 2° C  8° C



ScyTek Laboratories, Inc.
205 South 600 West
Logan, UT 84321
U.S.A.

CE 

EC REP
Emergo Europe
Prinsessegracht 20
2514 AP The Hague, The Netherlands

Troubleshooting Guide

Overstaining:

1. Concentration of the primary antibody was too high or the incubation time was too long.
2. Temperature during incubation was too high.
3. Incubation time with link antibody or streptavidin/enzyme label was too long.

Nonspecific Background Staining:


1. Rinsing between steps was inadequate.
2. Tissue was allowed to dry with reagents on.
3. Folds in tissue trapped reagents.
4. Tissue contains endogenous peroxidase.
5. Tissue contains endogenous biotin.
6. Antigen migrated in tissue.
7. Excessive tissue adhesive on slides.
8. Inadequate blocking with protein block.


Weak Staining:

1. Primary antibody concentration was too low or incubation time was too short.
2. Reagents are past their expiration date.
3. Inadequate removal of wash water between steps, resulting in dilution of reagents.
4. Counterstain or mounting media were incompatible and dissolved the chromogen reaction product.
5. Room temperature was excessively cool.
6. The primary antibody does not recognize an antigen that survives fixation and embedding in high enough amounts.
7. Excessive incubation with protein block (Super Block).

References:


1. Kuyucu Y, Sencar L, Tap Ö, Mete UÖ. Investigation of the effects of vitamin D treatment on the ovarian AMH receptors in a polycystic ovary syndrome experimental model: an ultrastructural and immunohistochemical study. *Reproductive Biology*. 2020 Jan 8.
2. Avcioglu G, Ipteç BÖ, Akcan G, Görgün B, Fidan K, Carhan A, Yilmaz G, Kozaci LD. Effects of 1, 25-Dihydroxy vitamin D 3 on TNF- α induced inflammation in human chondrocytes and SW1353 cells: a possible role for toll-like receptors. *Molecular and cellular biochemistry*. 2020 Jan 1;464(1-2):131-42.
3. Çetin A, Biltekin B. Combining Ellagic Acid with Temozolomide Mediates the Cadherin Switch and Angiogenesis in a Glioblastoma Model. *World neurosurgery*. 2019 Dec 1;132:e178-84.
4. Günes-Bayir, A., Kocyigit, A., Güler, E. M., Bilgin, M. G., Ergün, İ. S., & Dadak, A. (2018). Effects of carvacrol on human fibroblast (WS-1) and gastric adenocarcinoma (AGS) cells in vitro and on Wistar rats in vivo. *Molecular and Cellular Biochemistry*, 448(1), 237–249. <https://doi.org/10.1007/s11010-018-3329-5>
5. Kuyucu Y, Çelik LS, Kendirlihan Ö, Tap Ö, Mete UÖ. Investigation of the uterine structural changes in the experimental model with polycystic ovary syndrome and effects of vitamin D treatment: An ultrastructural and immunohistochemical study. *Reproductive biology*. 2018 Mar 1;18(1):53-9.
6. ÖZDEN FO, SAKALLIOĞLU EE, SAKALLIOĞLU U, AYAS B, ERIŞGİN Z. Effects of grape seed extract on periodontal disease: an experimental study in rats. *Journal of Applied Oral Science*. 2017 Apr;25(2):121-9.

 Storage: 2° C  8° C



ScyTek Laboratories, Inc.
205 South 600 West
Logan, UT 84321
U.S.A.


Emergo Europe
Prinsessegracht 20
2514 AP The Hague, The Netherlands

P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 – Tel. (435) 755-9848 - Fax (435) 755-0015 - www.scytek.com

7. Mertoğlu C, Senel U, Cayli SE, Tas U, Küskü Kiraz Z, Özyurt H. Protective role of methylprednisolone and heparin in ischaemic-reperfusion injury of the rat testicle. *Andrologia*. 2016 Sep;48(7):737-44.
8. Koç, M., Kumral, Z. N. Ö., Özkan, N., Memi, G., Kaçar, Ö., Bilsel, S., ... Yeğen, B. Ç. (2014). Obestatin improves ischemia/reperfusion-induced renal injury in rats via its antioxidant and anti-apoptotic effects: Role of the nitric oxide. *Peptides*, 60, 23–31. <https://doi.org/10.1016/j.peptides.2014.07.019>
9. Uzar, E., Acar, A., Evliyaoğlu, O., Fırat, U., Kamasak, K., Göçmez, C., ... İlhan, A. (2012). The anti-oxidant and anti-apoptotic effects of nebivolol and zofenopril in a model of cerebral ischemia/reperfusion in rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 36(1), 22–28. <https://doi.org/10.1016/j.pnpbp.2011.08.011>
10. Yasoda, A., Ogawa, Y., Suda, M., Tamura, N., Mori, K., Sakuma, Y., ... Nakao, K. (1998). Natriuretic Peptide Regulation of Endochondral Ossification EVIDENCE FOR POSSIBLE ROLES OF THE C-TYPE NATRIURETIC PEPTIDE/GUANYLYL CYCLASE-B PATHWAY. *Journal of Biological Chemistry*, 273(19), 11695–11700. <https://doi.org/10.1074/jbc.273.19.11695>
11. Yoshida, T., Matsumoto, E.-I., Hanamura, N., Kalembeiyi, I., Katsuta, K., Ishihara, A., & Sakakura, T. (1997). Co-expression of tenascin and fibronectin in epithelial and stromal cells of benign lesions and ductal carcinomas in the human breast. *The Journal of Pathology*, 182(4), 421–428. [https://doi.org/10.1002/\(SICI\)1096-9896\(199708\)182:4<421::AID-PATH886>3.0.CO;2-U](https://doi.org/10.1002/(SICI)1096-9896(199708)182:4<421::AID-PATH886>3.0.CO;2-U)

Storage: 2° C



8° C



ScyTek Laboratories, Inc.
205 South 600 West
Logan, UT 84321
U.S.A.



EC REP

Emergo Europe
Prinsessegracht 20
2514 AP The Hague, The Netherlands