



# Ready-to-use IHC/ICC kit (Biotin free), One-Step HRP Polymer anti-Mouse, Rat & Rabbit IgG with DAB

(Catalog # K405-50; 50 Tests [0.1 ml/slide]; Store at 2-8°C)

#### I. Introduction:

Immunohistochemistry (IHC)/Immunocytochemistry (ICC) is the localization of antigens in tissue sections/cells by the use of labeled antibodies as specific reagents through antigen-antibody interactions that are visualized by a marker such as fluorescent dye, enzyme, radioactive element or colloidal gold. Several IHC techniques are commonly used such as labeled biotin secondary antibody streptavidin-peroxidase, HRP anti-HRP, ABC catalyzed signal amplification, polymer system (one or two steps) etc. to detect antigens in tissues and cells. BioVision's Ready-to-use IHC kit employs polymer technology that provides increased sensitivity and detection. Our one-Step antimouse, Rat and Rabbit IgG (H+L) is a biotin/avidin free system that stains membrane, cytoplasmic and nuclear antigens. It provides the user with a rapid and easy to use IHC detection system.

#### II. Application

• Immunohistochemistry (IHC) and Immunocytchemistry (ICC).

### III. Kit Contents:

Components	K405-50	Cap Code	Part Number
Peroxidase Block (H <sub>2</sub> O <sub>2</sub> )	5 ml	NM	K405-50-1
Protein Blocking solution	5 ml	Blue	K405-50-2
Primary Ab dilution buffer	7.5 ml	Red	K405-50-3
HRP-anti-Mouse, Rat & Rabbit Polymer	5 ml	Amber	K405-50-4
Reagent BS (buffer & substrate)	5 ml	Green	K405-50-5
Reagent C (conc. DAB chromogen)	1 ml	Red	K405-50-6

#### IV. User Supplied Reagents and Equipment:

Washing buffer, antigen retrievers, positive or negative control, primary antibody, counterstain and mounting medium.

#### V. Storage and Handling:

Store reagents at 2-8°C, do not freeze. Bring reagents to RT before using. Positive and negative controls should be run simultaneously with the test specimens.

# VI. IHC/ICC procedure for frozen, paraffin sections and cell smears:

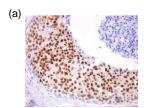
- 1. Deparafinize and hydrate tissue sections through xylene or other clearing agents and graded alcohols.
  - **Note**: For frozen sections or cell smears; use unfixed, acetone fixed or appropriate fixative for the antigen in question. For cell smears it may be necessary to permeabilize the cells by detergent Triton or NP40 (0.1-0.2% in PBS for 10 min.). Tween20, Saponin, Digitonin or leucoperm (0.2-0.5% in PBS for 10-30 min.) are suitable for cytoplasmic or plasma membrane antigens.
- 2. Wash 2-3 times with distilled or deionized water.
- 3. Incubate sections/cell smear with Peroxidase Block (clear cap) for 5-10 minutes at room temp. Wash with distilled water 3X. **Note:** If antigen retriever (Trypsin AR-6541, Pronase AR-6542, Pepsin AR-6543, Citrate buffer AR-6544, Buffer w EDTA pH 8.5 AR-6545, Tris buffer pH 10 AR-6546) is required, it can be applied at this step.
- 4. Wash slide with PBS or Tris saline buffer (with 0.02-0.05% nonionic detergent, Triton X100, Tween 20 or NP-40) 3X.
- 5. Incubate sections/ cell smear in Protein blocking solution (blue cap), for 5-10 minutes at RT.
- 6. Incubate sections/cell smear with primary antibody (not supplied, only buffer is supplied for dilution) for 20-30 minutes at RT. The primary antibody dilution buffer supplied can also be used as a negative control.
- 7. Wash slide with PBS 5-7X. Incubate with One-Step HRP polymer (brown cap) for 20-30 minutes at RT.
- 8. Wash slide 5-7 times with PBS or TBS preferably with 0.025-0.05% non-ionic detergent (Triton x-100 or Tween- 20 or NP-40) without sodium azide.
  - Caution: Peroxidase reagents are destroyed by sodium azide and should be avoided in all buffers and regents.
- 9. Wash slide with deionized or distilled for 2-3X.
- 10. In a test tube, add 1 ml of Reagent BS & 50 μl of Reagent C (DAB chromogen). Mix well. (This ready-to-use DAB reagent is good for 7-8 hr.)
- 11. Add few drops of ready to use DAB reagent on tissue slides & wait for 6-10 minutes at RT. (Higher temp. 37°C can also be used for DAB chromogen, however the incubation time should be determined by the individual lab)
- 12. Wash 5-7X with PBS, followed by rinsing with distilled or deionized water.
- 13. Incubate with counterstain compatible with DAB (Not supplied) for 30-60 sec. We prefer hematoxylin.
- 14. Wash slide with distilled/deionized water.
- 15. Mount with appropriate mounting medium e.g. SuperImmunoHistoMount™ (BV Cat. No: 1219-30)

Note: These are guidelines, the optimum incubation times for these reagents and reactions should be determined by the individual user.



#### Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM Tel 0032 16 58 90 45 info@gentaur.com





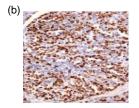




Figure: Formalin fixed breast cancer tissue (a & c) & breast tissue (b) stained with chromogen DAB & counterstained with Hematoxylin. Primary Antibody: anti-estrogen receptor (a), anti-Ki-67 (b) & anti-progesterone receptor (c).

**RELATED PRODUCTS:**FluorLast<sup>™</sup> Mounting Media
FluorLast<sup>™</sup> with DAPI FluorLast with DABCO
FluorLast<sup>TM</sup> with DAPI & DABCO
SuperMount<sup>TM</sup> Mounting Media
SuperImmunoHistoMount<sup>TM</sup> Cytosol/Particulate Separation Kit FractionPREP<sup>TM</sup> Cell Fractionation Kit Membrane Protein Extraction Kit Mitochondrial/Cytosol Fractionation Kit Mammalian Cell Extraction Kit Nuclear/Cytosol Fractionation Kit

## **Antibodies & Supporting Tools**

Primary Antibodies Secondary Antibodies **Blocking Peptides** Marker Antibodies Fluorescent Protein Antibodies Tag Antibodies Loading Control Antibodies

FOR RESEARCH USE ONLY! Not to be used on humans.