Kreative Technolabs

MOUSE AND HUMAN KI67 IMMUNOHISTOCHEMISTRY KIT

Ready-to-use IHC/ICC kit (Biotin free),

One-Step HRP Polymer anti-Mouse, Rat & Rabbit IgG with DAB

(Catalog # KT-RUK-K1001; 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. Kreative's Ready-to-use IHC kit employs polymer technology that provides increased sensitivity and detection. Our one-Step anti- mouse, 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
Peroxidase Block (H_2O_2)	5 ml	NM
Protein Blocking solution Primary Ab dilution buffer	5 ml 7.5 ml	Blue Red
HRP-anti-Mouse, Rat & Rabbit Polymer	5 ml	Amber
Reagent BS (buffer & substrate)	5 ml	Green
Reagent C (conc. DAB chromogen)	1 ml	Red

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, Pronase, Pepsin, Citrate buffer, Buffer w EDTA pH 8.5, Tris buffer pH 10) 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.

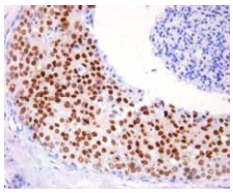
10. In a test tube, add 1 ml of Reagent BS & 50 µl of Reagent C (DAB chromogen). Mix well. (This ready-to-use DAB reagent is good for 7- 8 hr.)

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.

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

(b)



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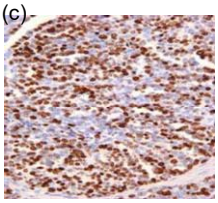

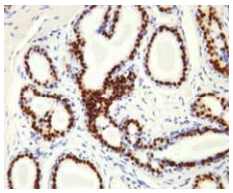

Figure: Formalin fixed breast cancer tissue (a & c) & breast tissue (b) stained with chromogen DAB & counterstained with Hematoxylin. Primary Antibody: anti-estrogen receptor (a), anti-Ki-67 (b) & anti-progesterone receptor (c).

For In-Vitro Diagnostic Use. Not For Human Or Animal Consumption.