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Human IKB alpha Ready-To-Use IHC Kit

Cat.No: IHC0372H
Applications: IHC-P
Reactivity: Human
Size: 50T
Assay type: Immunohistochemistry
Sample type: FFPE tissue
General Information:

Number	Component	Size	Concentration	Storage
1	PBS Buffer (powder)	2 L×2	20x	RT
2	Antigen Retrieval Buffer	20 ml	100x	2-8°C
3	Endogenous Peroxidase Blocking Buffer	3 ml	RTU	2-8°C, protect from light
4	Blocking Buffer	3 ml	RTU	2-8°C
5	Primary Antibody (Human IKB alpha Recombinant Rabbit mAb)	6 ml	RTU	2-8°C
6	Secondary Antibody (Goat Anti-Rabbit IgG H&L, HRP conjugated)	6 ml	RTU	2-8°C
7	Chromogen Component A	0.3 ml	RTU	-20°C,protect from light
8	Chromogen Component B	0.3 ml	RTU	-20°C
9	Counter Staining Reagent	5 ml	RTU	RT
10	Mounting Media	5 ml	RTU	RT
11	Control slide (Human kidney)	1 slide	RTU	RT
12	Datasheet	1 copy		

Storage and Stability: Please store components at the temperatures indicated on the individual tube labels. The kit is stable for 6 months from the date of receipt.

Immunohistochemistry Protocol:

1. **Deparaffinization And Rehydration**

Immerse slides in fresh xylene for 15 minutes and then repeat two more times using separate containers. Immerse slides sequentially in 100%, 95%, 90%, 80%, and 70% ethanol solutions for 5 minutes each. Rinse slides 3 times with distilled water for 5 minutes each.

2. **Antigen Retrieval**

Add 100× **Antigen Retrieval Buffer** into distilled water to prepare a 1×solution. Boil slides in 1×solution at 95°C-100°C for 15 minutes. Move the slides to 1×solution at room temperature (RT) and allow them to stand for 20 minutes. Rinse 3 times with **PBS Buffer** (dissolve the powder in 2L distilled water) for 5 minutes each.

3. **Block Endogenous Peroxidase**

Drain the liquid off the slides and then use a hydrophobic IHC pen to draw circles on the slides around tissue sections. Add 2-4 drops of **Endogenous Peroxidase Blocking Buffer** directly on slides, covering the whole tissue and block slides for 15 minutes at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

4. **Serum Blocking**

Block with 2-4 drops of **Blocking Buffer** for 20 minutes at RT.

5. **Primary Antibody Incubation**

Drain blocking buffer from slides. Incubate slides with 2-4 drops of **Human IKB alpha Recombinant Rabbit mAb** overnight at 4°C or 1-2 hours at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

6. **Secondary Antibody Incubation**

Incubate slides with 2-4 drops of **Goat Anti-Rabbit IgG H&L, HRP conjugated** for 1-2 hours at RT. Rinse slides 3 times with **PBS Buffer** for 5 minutes each.

7. **Signal Development**

Remove residual liquid around the tissue section. Add 50ul fresh **DAB Buffer** (**Chromogen Component A : Chromogen Component B : PBS Buffer=1:1:18**) to cover the tissue. Monitor the reaction under the microscope until a brown color is visible (approximate 3-5 minutes at RT). Stop reaction immediately by rinsing with distilled water. Rinse slides 3 times with distilled water for 5 minutes each.

8. **Counterstain**

Counterstain with an appropriate amount of **Counter Staining Reagent** for 3-5 minutes at RT. Rinse slides with distilled water for 5 minutes. Use 2-4 drops of **Differentiation reagent** to cover the tissue for 30 seconds. Rinse slides twice with distilled water for 5 minutes each.

9. **Dehydration Sheet**

Immerse slides sequentially in 70%, 80%, 90%, 95%, and 100% ethanol for 5 minutes each at RT. Immerse slides in 2 changes of fresh xylene, 15 minutes each. Drop some **Mounting Media** on the tissue. Mount coverslips.

Notes:

1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
2. Do not allow slides to dry at any time during this procedure.

3. Please don't replace the matching reagents in this product with other manufacturers' products.

4. As DAB is a carcinogen, please take necessary precautions.

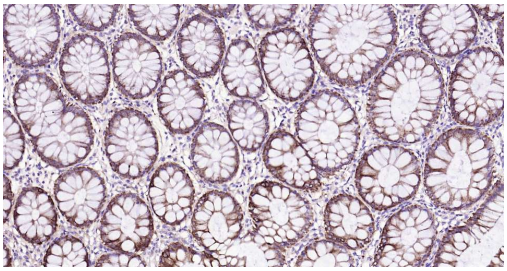
5. PBS (reagent 1) can be stored for one week at 4°C after preparation; The antigen retrieval buffer (1×reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

Please cite this product as " IHC0372H, Bioss Antibodies". Citation example: " Human Tissue sections using NFKBIA IHC Kit (IHC0372H, Bioss Antibodies) were stained for NFKBIA according to the manufacturer's instructions."

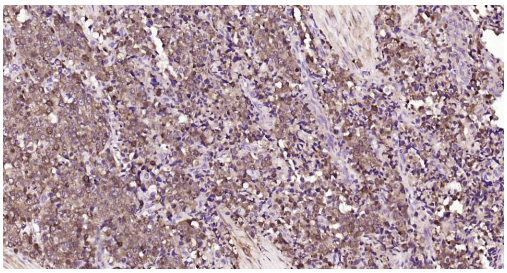
Introduction:

IkB-alpha is a 40 kDa protein that functions to inhibit NF- kappaB activity. The inhibition occurs via protein-protein interaction between I kappaB proteins and NF- kappaB dimers in the cytosol. The interaction of I kappa B-alpha with NF- kappaB masks the nuclear localization sequence of NF- kappaB, preventing NF- kappaB translocation to the nucleus. A variety of stimuli can activate gene expression by liberating NF- kappaB through the degradation of I kappaB alpha. These stimuli include the proinflammatory cytokines TNF-alpha and IL-1 beta, chemokines, PMA, growth factors, LPS, UV irradiation, viral infection, as well as various chemical and physical stresses. In humans, the gene is located on the q arm of chromosome 14. Activation of NFkB requires that IkB be phosphorylated on specific serine residues, which results in targeted degradation of IkB. IkB kinase alpha (IKK alpha), previously designated CHUK, interacts with IkB-alpha and specifically phosphorylates IkB-alpha on the sites that trigger its degradation Serines 32 and 36. IKK alpha appears to be critical for NFkB activation in response to proinflammatory cytokines. Phosphorylation of IkB by IKK alpha is stimulated by the NFkB inducing kinase (NIK), which itself is a central regulator for NFkB activation in response to TNF and IL-1. The functional IKK complex contains three subunits, IKK alpha, IKK beta and IKK gamma, and each appear to make essential contributions to IkB phosphorylation.

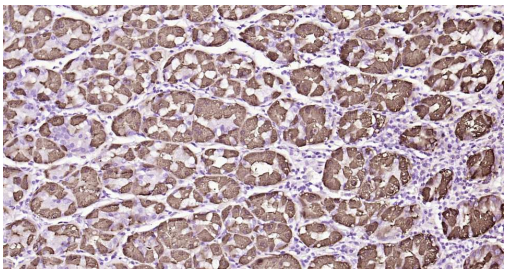
Validation Data



Immunohistochemical analysis of paraffin embedded human colon tissue slide using IHC0372H (Human IKB alpha Kit).



Immunohistochemical analysis of paraffin embedded human prostate tumor tissue slide using IHC0372H (Human IKB alpha Kit).



Immunohistochemical analysis of paraffin embedded human stomach tissue slide using IHC0372H (Human IKB alpha Kit).