

## Human MtTF1 Ready-To-Use IHC Kit

Cat.No: IHC0443H  
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 MtTF1 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 MtTF1 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.

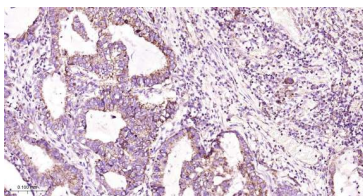
3. Please don't replace the matching reagents in this product with other manufacturers' products.
4. As DAB is a carcinogen, please take necessary precautions.
5. PBS (reagent 1) can be stored for one week at 4°C after preparation; The antigen retrieval buffer (1× reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

***Please cite this product as "IHC0443H, Bioss Antibodies". Citation example: "Human Tissue sections using TFAM IHC Kit (IHC0443H, Bioss Antibodies) were stained for TFAM according to the manufacturer's instructions."***

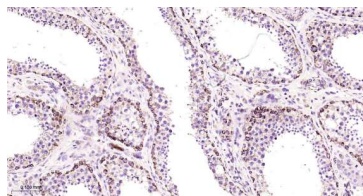
## Introduction:

Mitochondrial transcription factor A (TFAM) is a crucial protein encoded by nuclear genes, but it is transported from the cytoplasm to mitochondria where it plays a key role in the maintenance and expression of mitochondrial DNA (mtDNA). It regulates the replication and transcription of mtDNA, ensuring the proper functioning of mitochondrial processes. Structurally, TFAM contains a molecular region known as the LC3 interacting region (LIR) motif, which allows it to bind to autophagy proteins such as LC3. This interaction is essential for the autolysosomal pathway, helping to eliminate leaked mtDNA and limit inflammation. TFAM levels vary across tissues, with notable effects on mitochondrial function; high levels can repress mtDNA expression in certain tissues, such as skeletal muscle, and contribute to deficiencies in oxidative phosphorylation (OXPHOS). Conversely, in other tissues like the heart, increased mtDNA copy number maintains a balanced TFAM-to-mtDNA ratio, preserving OXPHOS capacity. TFAM is integral to the packaging, stability, and replication of the mitochondrial genome, and disruptions in TFAM can have severe effects, including implications in neurodegeneration and other diseases related to mtDNA stress.

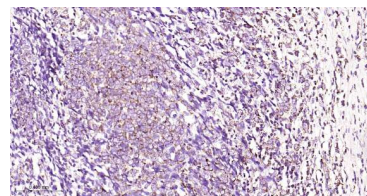
## Validation Data



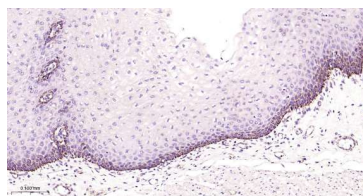
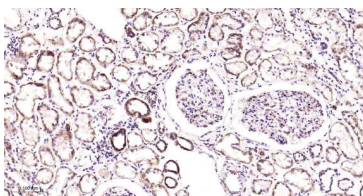
Immunohistochemical analysis of paraffin embedded human lung cancer tissue slide using IHC0443H (Human MtTF1 IHC Kit).



Immunohistochemical analysis of paraffin embedded human testis tissue slide using IHC0443H (Human MtTF1 IHC Kit).



Immunohistochemical analysis of paraffin embedded human thyroid carcinoma tissue slide using IHC0443H (Human MtTF1 IHC Kit).



Immunohistochemical analysis of paraffin  
embedded human kidney tissue slide using  
IHC0443H (Human MtTF1 IHC Kit).

Immunohistochemical analysis of paraffin  
embedded human esophagus tissue slide using  
IHC0443H (Human MtTF1 IHC Kit).