

Human Placental alkaline phosphatase (PLAP) Ready-To-Use IHC Kit

Cat.No: IHC0432H
 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 Placental alkaline phosphatase (PLAP) 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 placenta)	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 Placental alkaline phosphatase (PLAP) 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:

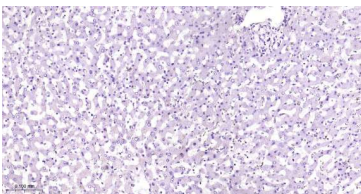
1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
2. Do not allow slides to dry at any time during this procedure.
3. Please don't replace the matching reagents in this product with other manufacturers' products.
4. As DAB is a carcinogen, 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 "IHC0432H, Bioss Antibodies". Citation example: "Human Tissue sections using ALPP IHC Kit (IHC0432H, Bioss Antibodies) were stained for ALPP according to the manufacturer's instructions."

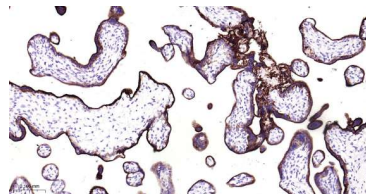
Introduction:

Placental Alkaline Phosphatase plays an important role in the regulation of specific inflammatory disease processes. There are at least four distinct but related alkaline phosphatases: intestinal, placental, placental-like, and liver/bone/kidney for this form of alkaline phosphatase have been well characterized. Placental Alkaline Phosphatase reacts with a membrane-bound isoenzyme (Regan and Nagao type) of Placental Alkaline Phosphatase (PLAP) occurring in the placenta during the 3rd trimester of gestation. Placental Alkaline Phosphatase is useful in the identification of testicular germ cell tumors. Unlike germ cell tumors, PLAP-positive somatic cell tumors uniformly express epithelial membrane antigen (EMA). A proposed function of Placental Alkaline Phosphatase is matrix mineralization; however, mice that lack a functional form of this enzyme show normal skeletal development. Placental Alkaline Phosphatase has been linked directly to hypophosphatasia, a disorder that is characterized by hypercalcemia and includes skeletal defects. The character of hypophosphatasia can vary, however, depending on the specific mutation since this determines age of onset and severity of symptoms.

Validation Data



Immunohistochemical analysis of paraffin embedded human liver tissue slide using IHC0432H (Human Placental alkaline phosphatase (PLAP) IHC Kit).



Immunohistochemical analysis of paraffin embedded human placenta tissue slide using IHC0432H (Human Placental alkaline phosphatase (PLAP) IHC Kit).