

## Mouse Phospho-PERK Ready-To-Use IHC Kit

**Cat. No.: IHC0120M**

**Size: 50T (including a control slide)**

**Sample Type: FFPE tissue**

**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.**

### 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   |
| 4      | Blocking Buffer                                   | 3 ml    | RTU           | 2-8°C   |
| 5      | Primary Antibody (Mouse Phospho-PERK Rabbit pAb)  | 6 ml    | RTU           | 2-8°C   |
| 6      | Secondary Antibody (HRP-Goat anti-Rabbit IgG pAb) | 6 ml    | RTU           | 2-8°C   |
| 7      | Chromogen Component A                             | 0.3 ml  | RTU           | -20°C   |
| 8      | Chromogen Component B                             | 0.3 ml  | RTU           | -20°C   |
| 9      | Counter Staining Reagent                          | 5 ml    | RTU           | RT      |
| 10     | Differentiation Reagent                           | 6 ml    | RTU           | RT      |
| 11     | Mounting Media                                    | 5 ml    | RTU           | RT      |
| 12     | Control slide (Mouse testis)                      | 1 slide | RTU           | RT      |
| 13     | Datasheet   | 1 copy  |               |         |

### Background

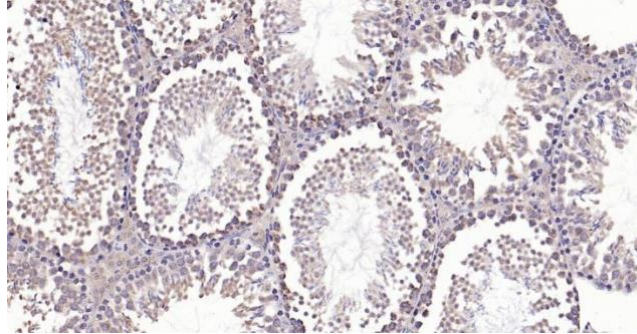
PERK, a member of the GCN2 subfamily of Ser/Thr protein kinases, phosphorylates the alpha subunit of eukaryotic translation-initiation factor 2 (EIF2), leading to its inactivation and thus to a rapid reduction of translational initiation and repression of global protein synthesis. It likely serves as a critical effector of unfolded protein response (UPR)-induced G1 growth arrest due to the loss of cyclin D1. Perturbation in protein folding in the endoplasmic reticulum (ER) promotes reversible dissociation from HSPA5/BIP and oligomerization, resulting in transautophosphorylation and kinase activity induction. Expression of this Type I membrane protein is ubiquitous, with highest levels seen in secretory tissues. Defects in EIF2AK3 are the cause of Wolcott-Rallison syndrome (WRS), also known as multiple epiphyseal dysplasia with early-onset diabetes mellitus. WRS is a rare autosomal recessive disorder, characterized by permanent neonatal or early infancy insulin-dependent diabetes and, at a later age, epiphyseal dysplasia, osteoporosis, growth retardation and other multisystem manifestations, such as hepatic and renal dysfunctions, mental retardation and cardiovascular abnormalities.

### Synonyms

p-PERK(Thr980); PERK(Phospho Thr980); PERK(Phospho T980); mo(Thr980)/hu (Thr982)/rat (Thr974); HRI; HsPEK; Pancreatic eIF2-alpha kinase; PEK; PRKR like endoplasmic reticulum kinase; WRS; DKFZp781H1925; EC 2.7.11.1; EIF2AK3; Eukaryotic translation initiation factor 2 alpha kinase 3; Heme regulated EIF2 alpha kinase.

**Important Note: This product as supplied is intended for research use only, not for use in human, therapeutic or diagnostic applications.**

## Validation Data



Immunohistochemical analysis of paraffin embedded mouse testis tissue slide using IHC0120M (Mouse Phospho-PERK IHC Kit).

## 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 **Mouse Phospho-PERK Rabbit pAb** 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 **HRP-Goat anti-Rabbit IgG pAb** 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.

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## 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 " IHC0120M, Bioss Antibodies".**

**Citation example: "Mouse tissue sections using Mouse Phospho-PERK IHC Kit (IHC0120M, Bioss Antibodies) were stained for Phospho-PERK according to the manufacturer's instructions.**

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