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# **RPS6KB1 ELISA Kit**



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Quantity:	96 tests	
Target:	RPS6KB1	
Binding Specificity:	pSer424, pThr421, total	
Reactivity:	Human, Mouse, Rat	
Method Type:	Sandwich ELISA	
Application:	ELISA	
Product Details		
Purpose:	Human/Mouse/Rat Phospho-P70S6K (T421/S424) and Total P70S6K ELISA Kit. This assay semi-quantitatively measures phophorylated P70S6K (Thr421/S424) and Total P70S6K in lysate samples.	
Sample Type:	Cell Lysate, Tissue Lysate	
Analytical Method:	Semi-Quantitative	
Detection Method:	Colorimetric	
Specificity:	The antibody pair provided in this kit recognizes human, mouse, and rat p70S6 Kinase phosphorylated at sites Threonine-421 and/or Serine-424 as well as total (pan) p70S6 Kinase.	
Characteristics:	<ul> <li>Simultaneously measure Phosphorylated protein and pan protein in one experiment (for normalization purpose)</li> <li>Screen numerous different cell lysates without performing a Western Blot analysis</li> <li>Minimal hands-on time, convenient, and non-radioactive material</li> </ul>	
Components:	Pre-Coated 96-well Strip Microplate	

- · Wash Buffer
- · Anti-Phospho Antibody
- · Anti-Pan Antibody
- · HRP-Conjugated Secondary Antibody
- · Streptavidin-Conjugated HRP
- · Assay Diluent
- · TMB One-Step Substrate
- · Stop Solution
- · Lysis Buffer
- · Positive Control Sample

### Material not included:

- · Distilled or deionized water
- · 100 mL and 1 liter graduated cylinders
- Tubes to prepare sample dilutions
- · Protease and Phosphatase inhibitors
- Precision pipettes to deliver 2 µL to 1 mL volumes
- · Adjustable 1-25 mL pipettes for reagent preparation
- · Benchtop rocker or shaker
- Microplate reader capable of measuring absorbance at 450 nm

## **Target Details**

Target:	RPS6KB1
Alternative Name:	P70S6K (RPS6KB1 Products)
Background:	P70S6 kinase (P70S6K) phosphorylated at T421 and/or S424 and total (pan) p70S6 kinase
Gene ID:	6198
UniProt:	P23443
Pathways:	PI3K-Akt Signaling, RTK Signaling, AMPK Signaling, Regulation of Cell Size, Skeletal Muscle Fiber Development, Feeding Behaviour, G-protein mediated Events, Smooth Muscle Cell Migration, Interaction of EGFR with phospholipase C-gamma, Warburg Effect

# **Application Details**

Sample Volume:	100 μL
Plate:	Pre-coated
Protocol:	<ol> <li>Prepare all reagents and samples as instructed in the manual.</li> <li>Add 100 μL of sample or positive control to each well.</li> </ol>

- 3. Incubate 2.5 h at RT or O/N at 4 °C.
- 4. Add 100 µL of prepared primary antibody to each well.
- 5. Incubate 1 h at RT.
- 6. Add 100  $\mu L$  of prepared 1X HRP-Streptavidin to each well.
- 7. Incubate 1 h at RT.
- 8. Add 100 µL of TMB One-Step Substrate Reagent to each well.
- 9. Incubate 30 min at RT.
- 10. Add 50 µL of Stop Solution to each well.
- 11. Read at 450 nm immediately.

### Assay Procedure:

Prepare all reagents and samples as instructed in the manual.

Add 100 µL of sample or positive control to each well.

Incubate 2.5 h at RT or O/N at 4 °C.

Add 100 µL of prepared primary antibody to each well.

Incubate 1 h at RT.

Add 100 µL of prepared 1X HRP-Streptavidin to each well.

Incubate 1 h at RT.

Add 100 µL of TMB One-Step Substrate Reagent to each well.

Incubate 30 min at RT.

Add 50 µL of Stop Solution to each well.

Read at 450 nm immediately.

Restrictions:

For Research Use only

### Handling

Storage:	-20 °C
Storage Comment:	Upon receipt, the kit should be stored at -20 °C. Please use within 6 months from the date of
	shipment. After initial use, Wash Buffer Concentrate (Item B), Assay Diluent (Item E), TMB One-
	Step Substrate Reagent (Item H), HRP-Streptavidin (Item G), Stop Solution (Item I) and Cell
	Lysate Buffer (Item J) should be stored at 4 °C to avoid repeated freeze-thaw cycles. Return
	unused wells to the pouch containing desiccant pack, reseal along entire edge and store at -20
	°C. Reconstituted Positive Control (Item K) should be stored at -70 °C.

Expiry Date:

6 months