POLYCLONAL ANTIBODY

Anti-CREB [pSerpSer\(^{129/133}\)] Phosphospecific Antibody, Unconjugated

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Isotype:</th>
<th>Quantity:</th>
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<tbody>
<tr>
<td>AT-7098</td>
<td>Rabbit IgG</td>
<td>100 μL</td>
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**BACKGROUND:**
cAMP responsive element binding protein (CREB) is a 43 kDa leucine zipper transcription factor that stimulates transcription and gene expression after it is phosphorylated on serine residues 129 and 133. CREB plays an important role in development, glucose homeostasis and growth-factor-dependent cell survival, and is implicated in learning and memory. The phosphorylation of CREB at GSK-3β and PKA sites (serines 129 and 133, respectively) is essential for its transcriptional activity.

**PRODUCT:**
Rabbit polyclonal immunoglobulin in Dulbecco’s phosphate buffered saline (without Mg\(^{2+}\) and Ca\(^{2+}\), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier. 0.05% sodium azide.

**IMMUNOGEN:**
The antiserum was produced against a chemically synthesized phosphopeptide derived from a region of human CREB that contains serines 129 and 133. The sequence is conserved in mouse and rat.

**PURIFICATION:**
Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated CREB. The final product is generated by affinity chromatography using a CREB-derived peptide that is phosphorylated at serines 129 and 133.

**SPECIFICITY:**
Human and mouse CREB. Rat CREB (100% homologous) has not been tested, but is expected to react.

**APPLICATIONS:**
The antibody has been used in Western blotting. For Western blotting applications, we recommend using the antibody at a 1:1000 starting dilution. The optimal antibody concentration should be determined empirically for each specific application.

**STORAGE:**
Store at \(-20^\circ\)C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at \(-20^\circ\)C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.

**POSITIVE CONTROL:**
NIH3T3 cells +/- PDGF, Y-1 cells.

**REFERENCES:**


**Peptide Competition and Phosphatase Treatment**

Extracts of NIH3T3 cells untreated (1) or treated with 50ng/mL PDGF for 15 minutes (2-6) were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. The membrane was blocked with a 5% BSA-TBST buffer for one hour at room temperature and either left untreated (1-5) or treated with lambda (λ) phosphatase (6), then incubated with the CREB [pSerpSer^{129/133}] antibody for two hours at room temperature in a 3% BSA-TBST buffer, following prior incubation with: no peptide (1, 2, 6), the non-phosphopeptide corresponding to the phosphopeptide immunogen (3), a generic phosphoserine-containing peptide (4), or the phosphopeptide immunogen (5). After washing, the membrane was incubated with goat F(ab’)_2 anti-rabbit IgG HRP conjugate and signals were detected using the Pierce SuperSignal™ method. The data show that only the phosphopeptide corresponding to CREB [pSerpSer^{129/133}] blocks the antibody signal, demonstrating the specificity of the antibody. The data also show that phosphatase stripping eliminates the signal, further verifying that the antibody is phospho-specific.