FastScan™ Phospho-Bad (Ser112) ELISA Kit

1 Kit (96 assays)

For Research Use Only. Not For Use In Diagnostic Procedures.

Species Cross-Reactivity: H, M, R, Mk

Description: The FastScan™ Phospho-Bad (Ser112) ELISA Kit detects endogenous levels of Bad when phosphorylated at Ser112. To perform the assay, sample is incubated with a capture antibody conjugated with a proprietary tag and a second detection antibody linked to HRP, forming a sandwich with phospho-Bad (Ser112) in solution. This entire complex is immobilized to the plate via an anti-tag antibody. The wells are then washed to remove unbound material. TMB is then added. The magnitude of observed signal is proportional to the quantity of phospho-Bad (Ser112).

*Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: The FastScan™ Phospho-Bad (Ser112) ELISA Kit detects endogenous levels of Bad when phosphorylated at Ser112 as shown in Figure 1. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Background: Bad is a proapoptotic member of the Bcl-2 family that promotes cell death by displacing Bax from binding to Bcl-2 and Bcl-xL (1,2). Survival factors, such as IL-3, inhibit the apoptotic activity of Bad by activating intracellular signaling pathways that result in the phosphorylation of Bad at Ser112 and Ser136 (2). Akt phosphorylates Bad at Ser136 to promote cell survival (3,4). Bad is phosphorylated at Ser112 both in vivo and in vitro by p90RSK (5,6) and mitochondria-anchored PKA (7). Phosphorylation at Ser155 in the BH3 domain by PKA plays a critical role in blocking the dimerization of Bad and Bcl-xL (8-10).

<table>
<thead>
<tr>
<th>Product Includes</th>
<th>Item #</th>
<th>Kit Quantity</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>FastScan™ ELISA Microwell Strip Plate*</td>
<td>53257</td>
<td>96 tests</td>
<td></td>
</tr>
<tr>
<td>Bad Rabbit Capture mAb</td>
<td>99068</td>
<td>1 each</td>
<td>Green (Lyophilized)</td>
</tr>
<tr>
<td>Phospho-Bad (Ser112) Rabbit HRP-linked mAb</td>
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<td>1 each</td>
<td>Red (Lyophilized)</td>
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<tr>
<td>FastScan™ ELISA Capture Antibody Diluent</td>
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<td>3 ml</td>
<td>Green</td>
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<tr>
<td>FastScan™ ELISA HRP Antibody Diluent</td>
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<td>3 ml</td>
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</tr>
<tr>
<td>TMB Substrate</td>
<td>7004</td>
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<tr>
<td>STOP Solution</td>
<td>7002</td>
<td>11 ml</td>
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<tr>
<td>Sealing Tape</td>
<td>54503</td>
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<tr>
<td>ELISA Wash Buffer (20X)</td>
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<td>25 ml</td>
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<tr>
<td>FastScan™ ELISA Cell Extraction Buffer (5X)</td>
<td>69905</td>
<td>10 ml</td>
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<tr>
<td>FastScan™ ELISA Cell Extraction Enhancer Solution (50X)</td>
<td>25243</td>
<td>1 ml</td>
<td></td>
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<tr>
<td>FastScan™ ELISA Kit #30605 Positive Control Type 1</td>
<td>40292</td>
<td>1 each</td>
<td></td>
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</tbody>
</table>

* 12 8-well modules — Each module is designed to break apart for 8 tests.

Background References:


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A Solutions and Reagents

NOTE: Prepare solutions with deionized/purified water or equivalent. Prepare only as much reagent as needed on the day of the experiment.

1. FastScan™ ELISA Microwell Strip Plate, 96 well (#53257): Bring all to room temperature before opening bag/use. Unused microwell strips should be returned to the original re-sealable bag containing the desiccant pack and stored at 4°C.

2. 1X ELISA Wash Buffer: Prepare by diluting ELISA Wash Buffer (20X) (included in each kit) 1X with deionized water.

3. 1X Cell Extraction Buffer: Prepare by diluting FastScan™ ELISA Cell Extraction Buffer (5X) #69905 and FastScan™ ELISA Cell Extraction Enhancer Solution (50X) #25243 to 1X with deionized water. This buffer can be stored at 4°C for short-term use (1-2 weeks). To make 10 mL 1X Cell Extraction Buffer, combine 7.8 mL deionized water, 2 mL FastScan™ ELISA Cell Extraction Buffer (5X), and 200 μL FastScan™ ELISA Cell Extraction Enhancer Solution (50X). Alternatively, Enhancer Solution may be added to the Cell Extraction Buffer after extraction of cells or tissue. When using the 1X Cell Extraction Buffer as a sample diluent for the assay, it is recommended to equilibrate it to room temperature prior to use.

*IMPORTANT: The provided FastScan™ ELISA Cell Extraction Enhancer Solution (50X) may precipitate when stored at 4°C. To dissolve, warm briefly at 37°C.

B Preparing Cell Lysates

For adherent cells

1. Aspirate media when the culture reaches 80–90% confluence.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 mL ice-cold 1X Cell Extraction Buffer (recommended to supplement with protease inhibitors and additional phosphatase inhibitors as needed) to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 5 min (14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

For suspension cells

1. Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 x 106 viable cells/ml.
2. Wash once with ice-cold 1X PBS.
3. Cells harvested from 50 mL of growth media can be lysed in 2.0 mL of 1X Cell Extraction Buffer (recommended to supplement with protease inhibitors and additional phosphatase inhibitors as needed).
4. Sonicate lysates on ice.
5. Microcentrifuge for 5 min (14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

C Test Procedure

NOTE: Equilibrate all materials and prepared reagents to room temperature prior to running the assay.

1. Prepare all reagents as indicated above (Section A).
2. Samples should be undiluted or diluted with 1X Cell Extraction Buffer to a 2X protein concentration in order to achieve a final 1X protein concentration upon addition of the antibody cocktail. Individual datasheets for each kit provide a sensitivity curve that serves as a reference for selection of an appropriate starting lysate concentration. The sensitivity curve shows typical results across a range of lysate concentration points.
3. Add 50 μL of each sample or Positive Control to the appropriate wells.
4. Add 50 μL of the Antibody Cocktail to each well.
5. Seal the plate with the supplied sealing tape and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm (moderate agitation).
6. Gently remove the tape and wash wells:
   a. Discard plate contents into a receptacle.
   b. Wash 3 times* with 1X ELISA Wash Buffer, 200 μL each time for every well.
   c. After each wash, aspirate or decant from wells. Invert the plate and blot it against clean paper towels to remove the residual solution in each well, but do not allow wells to completely dry at any time.
   d. Clean the underside of all wells with a lint-free tissue.

   *NOTE: Certain FastScan™ ELISA Kits may require additional washes at this step. Any requirements for additional washes will be specifically noted in the product “Description” of the kit’s datasheet.

7. Add 100 μL of TMB Substrate to each well. Seal with tape and incubate the plate in the dark for 15 min at room temperature on a plate shaker (400 rpm, moderate agitation) or alternatively for 10 min at 37°C without shaking.
8. Add 100 μL of STOP Solution to each well. Shake gently for a few seconds. *NOTE: Initial color of positive reaction is blue, which changes to yellow upon addition of STOP Solution.
9. Read results:
   a. Visual Determination: Read within 30 min after adding STOP Solution.
   b. Spectrophotometric Determination: Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.