



cell signaling solutions

Certificate of Analysis

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CAKPAK Miniature Set
Catalog # 17-177
Lot # 23473

Table with 4 columns: Antibody, Anti-Human Focal Adhesion Kinase (p125FAK), Anti-Pyk2, and Anti ILK. Rows include Catalog #, Lot #, [Immunoblot], Amount, Physical Form, Storage & Stability, Immunogen, Purification, Known Species Cross-Reactivity, and Additional Applications.

Antibodies also available individually:

Table with 3 columns: Antibody, Catalog #, Pack Size. Rows: FAK (200µg), Pyk2 (250µg), ILK (100µg).

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1 μ g/ml aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (PBS-MLK) for 60 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with the indicated amount of primary antibody, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C. **Note:** The use of 0.05% Tween may enhance detection of p125^{FAK}.
4. Wash the nitrocellulose twice with water for 5 minutes. water for 5 minutes.
5. Incubate the nitrocellulose in the secondary reagent of choice in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water two times for 5 minutes.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).