

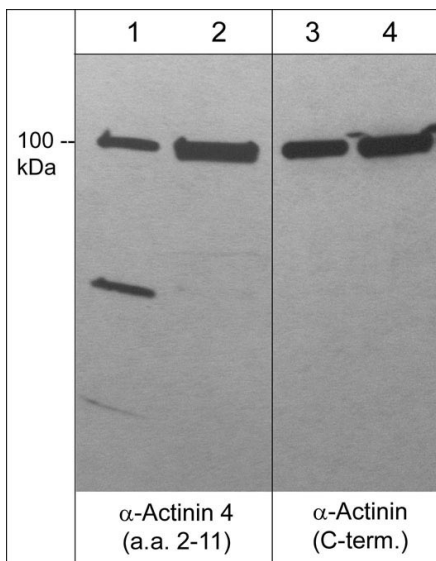
### Kit Summary

The α-Actinin 4 phospho-regulation antibody sampler kit can be used to examine phosphorylation of α-Actinin 4 at Tyr-4. The kit includes polyclonal anti-α-Actinin 4 antibody to monitor total expression levels of α-Actinins, peptides for blocking experiments, and HRP-conjugated secondary reagent for primary antibody detection.

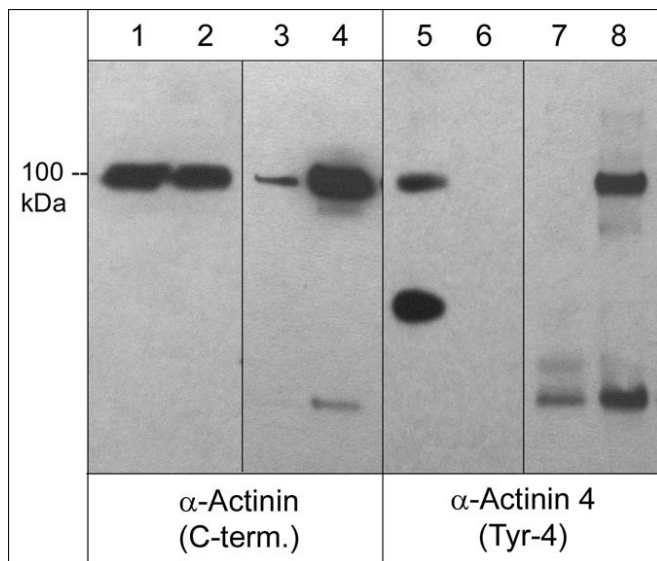
### Kit Components

| Cat. # | Description                             | Product Type | Size   | Applications    | Species Reactivity | WB Dilution |
|--------|---|--------------|--------|-----------------|--------------------|-------------|
| AP4271 | α-Actinin 4 (a.a. 2-11)                 | Rabbit pAb   | 50 µl  | WB, E           | Hu, Rt, Ms         | 1:500       |
| AX4275 | α-Actinin 4 (a.a. 2-11)                 | Peptide      | 50 µg  | AB, E           |                    |             |
| AP4241 | α-Actinin 4 (Tyr-4), phospho-specific   | Rabbit pAb   | 50 µl  | WB, E           | Hu, Rt, Ms         | 1:1000      |
| AX4245 | phospho-α-Actinin 4 (Tyr-4)             | Peptide      | 50 µg  | AB, E           |                    |             |
| RS3251 | Anti-Rabbit Ig Light-Chain Specific:HRP | Mouse mAb    | 100 µl | WB, E, ICC, IHC | Rb                 | 1:5000      |

Applications: WB = Western blot, E = ELISA, ICC = Immunocytochemistry, IP = Immunoprecipitation, IHC = Immunohistochemistry, FC = Flow Cytometry  
Species: H = Human, R = Rat, Ms = Mouse, C = Chicken, F = Fish, Fr = Frog, Rb = Rabbit



Western blot analysis of α-actinin in human A431 cells (lanes 1 & 3) and rabbit spleen fibroblasts (lanes 2 & 4). The blots were probed with rabbit polyclonal anti-α-actinin 4 (a.a. 2-11) or mouse monoclonal anti-α-actinin (C-terminal region).



Western blot analysis of α-actinin 4 in A431 cells stimulated with pervanadate (1 mM) for 30 min (lanes 1,2,5,6) or after immunoprecipitation using α-actinin (C-terminal region) antibody in the absence (lanes 3 & 7) or presence of pervanadate-treated A431 cell lysate (lanes 4 & 8). Some lanes of the blot were treated with alkaline phosphatase (lanes 2 & 6). The blots were probed with anti-α-actinin (C-terminal region) or anti-α-actinin 4 (Tyr-4).

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## Background

$\alpha$ -Actinins are widely expressed cytoskeletal proteins that cross-link actin filaments through antiparallel homodimers of the rod domains. Four  $\alpha$ -actinin genes have been discovered in humans with  $\alpha$ -actinin 1 and 4 being widely expressed in non-muscle cells.  $\alpha$ -Actinins contain three conserved domains that include an N-terminal actin binding domain, four spectrin-like repeats in the central region, and a C-terminal calmodulin binding domain.  $\alpha$ -Actinin cross-links the actin filament networks and associates the network to focal adhesion sites through binding of talin and vinculin.  $\alpha$ -Actinin 1 is phosphorylated at Tyr-12 by FAK, while  $\alpha$ -actinin 4 can be phosphorylated at Tyr-4 and Tyr-31 after EGF treatment. Tyr-4 and Tyr-31 phosphorylation inhibits actin binding and reduces actin-filament driven multinucleation in rat kidney cells. Thus, phosphorylation in  $\alpha$ -actinins may be important for regulating actin binding and actin cytoskeletal remodeling.

## Background References

- Izaguirre, G. et al. (2001) J Biol Chem. 276(31):28676.  
Shao, H. et al. (2010) J Biol Chem. 285(4):2591.

## Buffer and Storage

Rabbit polyclonal antibodies are supplied in phosphate-buffered saline, 50% glycerol, 1 mg/ml BSA, and 0.05% sodium azide. The secondary reagent is supplied in the same buffer without azide, and the peptides are supplied in PBS and 0.05% sodium azide. Store all at  $-20^{\circ}\text{C}$ . Stable for 1 year.

## Product Citations

| <u>Cat. #</u> | <u>Citation &amp; Application</u>  |
|---------------|--|
| AP4271        | Kojima, S. et al. (2014) Am J Nephrol. 39(1):36-45 (WB: human kidney cells)                                |
| AP4241        | Kojima, S. et al. (2014) Am J Nephrol. 39(1):36-45 (WB: human kidney cells)                                |
| RS3251        | Kawasaki, H. et al. (2013) World J Gastroenter. 19(17):2629. (WB, ICC: mouse intestinal myofibroblasts and |
| RS3251        | Estrada-Bernal, A. et al. (2011) J Neurooncol. 102:353. (Western blot)                                     |

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