Technical Data Sheet

# Purified Mouse Anti-AFAP

## Product Information

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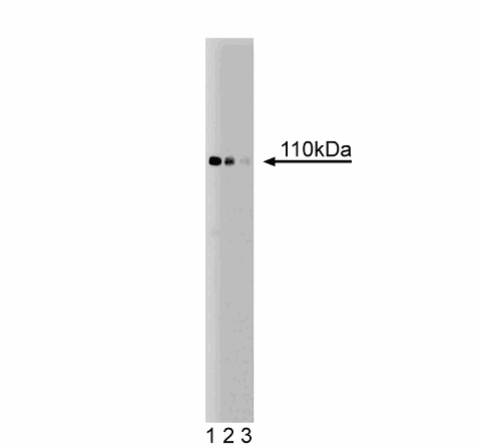
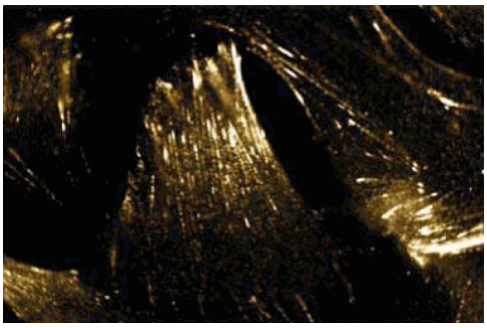
|---|---|
| **Material Number:** | **610200** |
| **Alternate Name:** | Actin Filament-Associated Protein-110 |
| **Size:** | 150 µg |
| **Concentration:** | 250 µg/ml |
| **Clone:** | 36/AFAP |
| **Immunogen:** | Chicken AFAP aa. 469-635 |
| **Isotype:** | Mouse IgG1 |
| **Reactivity:** | QC Testing: Chicken<br>Tested in Development: Human, Rat, Mouse |
| **Target MW:** | 110 kDa |
| **Storage Buffer:** | Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide. |

## Description

Chicken embryo fibroblasts (CEF) which are transformed by pp60[*src*] show an increase in the steady-state levels of tyrosine-phosphorylated proteins. One of these proteins is the Actin Filament-Associated Protein, AFAP110. This protein forms a stable complex with activated pp60[*src*] via the SH3 and SH2 domains of the src protein. However, tyrosine phosphorylation of AFAP requires only the intact src SH3 domain. AFAP is associated with actin stress fibers in normal CE cells, but following transformation by pp60[*src*], AFAP becomes localized in rosette-like structures known as podosomes. Several sequence motifs within AFAP110 are predicted to bind to SH2 and to SH3 domains. In vitro analysis of AFAP in normal cell extracts demonstrated that it binds less efficiently to Src SH2-containing fusion proteins than does AFAP from *src*-transformed cell extracts.




***Western blot analysis of AFAP on a SL-29 cell lysate (Chicken embryonic fibroblasts; ATCC CRL-1590).***
*Lane 1: 1:250, lane 2: 1:500, lane 3: 1:1000 dilution of the mouse anti-AFAP antibody.*



***Immunofluorescence staining of human fibroblasts.***

## Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at -20° C.

**BD Biosciences**

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## Application Notes

**Application**

| | |
|---|---|
| Western blot | Routinely Tested |
| Immunofluorescence | Tested During Development |
| Immunohistochemistry | Tested During Development |
| Immunoprecipitation | Not Recommended |

**Recommended Assay Procedure:**

***Western blot:*** Please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

## Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|---|---|---|---|
| 611478 | SL-29 Cell Lysate | 500 µg | (none) |
| 554002 | HRP Goat Anti-Mouse Ig | 1.0 ml | (none) |
| 554001 | FITC Goat Anti-Mouse Ig | 0.5 mg | Polyclonal |

## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

## References


Fincham VJ, Brunton VG, Frame MC. The SH3 domain directs acto-myosin-dependent targeting of v-Src to focal adhesions via phosphatidylinositol 3-kinase. *Mol Cell Biol.* 2000; 20(17):6518-6536.(Biology: Western blot)

Flynn DC, Leu TH, Reynolds AB, Parsons JT. Identification and sequence analysis of cDNAs encoding a 110-kilodalton actin filament-associated pp60src substrate. *Mol Cell Biol.* 1993; 13(12):7892-7900.(Biology)

Kanner SB, Reynolds AB, Vines RR, Parsons JT. Monoclonal antibodies to individual tyrosine-phosphorylated protein substrates of oncogene-encoded tyrosine kinases. *Proc Natl Acad Sci U S A.* 1990; 87(9):3328-3332.(Biology)

Kanner SB, Reynolds AB, Wang HC, Vines RR, Parsons JT. The SH2 and SH3 domains of pp60src direct stable association with tyrosine phosphorylated proteins p130 and p110. *EMBO J.* 1991; 10(7):1689-1698.(Biology)