Overview

Product name: Anti-Fibronectin antibody [IST-9] ab6328

Description: Mouse monoclonal [IST-9] to Fibronectin

Host species: Mouse

Specificity: This antibody reacts with an epitope (PEDGHELF) located in the ED-A sequence of cellular fibronectin (it therefore only detects the cFN and not extracellular FN (plasma FN)). (Liao et al.) The ED-A segment can be included or omitted from the molecule depending on the pattern of splicing of the mRNA precursors. In transformed or tumour derived cells the ED-A segment is about 10-times higher than in FN from normal fibroblasts, and it may therefore be a significant marker for malignancy (Borsi et al., and others).

Tested applications: Suitable for: WB, RIA, ELISA, IHC-Fr, ICC/IF, Other, Blocking, IHC-P

Species reactivity: Reacts with: Rat, Chicken, Cow, Dog, Human, Pig, Monkey

Predicted to work with: Mouse, Rabbit

Immunogen: Recombinant fragment corresponding to Human Fibronectin aa 1631-1721. Sequence of the type III repeats termed EIIIA (or ED-A).

Sequence:

NIDRPKGLAFTVDVDSDIAWESPGQVSRYRTYSS
PEDGIHELFPAP DGEEDTAELQ
GLRPGSEYTVSVVALHDDMESQPLGQTSTA

Database link: P02751

Epitope: Liao et al: Clone IST-9, binds to the lle43 and His44 residues within ED-A in a conformationally dependent fashion, implicating the loop region encompassing both residues as critical for mediating ED-A function. The conformational domain C-C'-E of rat EIIIA encompassing the His44 residue is crucial for constituting the IST-9 epitope.

Storage instructions
Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.

Storage buffer
Constituent: PBS

Purity
Immunogen affinity purified

Clonality
Monoclonal

Clone number
IST-9

Isotype
IgG1

Applications
Our Abpromise guarantee covers the use of ab6328 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 1 - 5 µg/ml.</td>
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<tr>
<td>RIA</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>AP</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>ELISA</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/1500.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration. Concentration of IST-9 in IHC-fr - depending on the system - is 0.1 to 2 micrograms per ml. IST-9 works very well using all iHC-fr procedures.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/200.</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>Blocking</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration. PubMed: 20643910</td>
</tr>
<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/100 - 1/200. Perform enzymatic antigen retrieval before commencing with IHC staining protocol. See Abreviews Concentration of IST-9 in IHC-P is - depending on the system - 2-10 micrograms per ml.</td>
</tr>
</tbody>
</table>

Target

Function
Fibronectins bind cell surfaces and various compounds including collagen, fibrin, heparin, DNA, and actin. Fibronectins are involved in cell adhesion, cell motility, opsonization, wound healing, and maintenance of cell shape. Involved in osteoblast compaction through the fibronectin fibrillogenesis cell-mediated matrix assembly process, essential for osteoblast mineralization. Participates in the regulation of type I collagen deposition by osteoblasts. Anastellin binds fibronectin and induces fibril formation. This fibronectin polymer, named superfibronectin, exhibits enhanced adhesive properties. Both anastellin and superfibronectin inhibit tumor growth, angiogenesis and metastasis. Anastellin activates p38 MAPK and inhibits lysophospholipid signaling.
**Tissue specificity**

Plasma FN (soluble dimeric form) is secreted by hepatocytes. Cellular FN (dimeric or cross-linked multimeric forms), made by fibroblasts, epithelial and other cell types, is deposited as fibrils in the extracellular matrix. Ugl-Y1, Ugl-Y2 and Ugl-Y3 are found in urine.

**Involvement in disease**

Glomerulopathy with fibronectin deposits 2

**Sequence similarities**

Contains 12 fibronectin type-I domains.
Contains 2 fibronectin type-II domains.
Contains 16 fibronectin type-III domains.

**Developmental stage**

Ugl-Y1, Ugl-Y2 and Ugl-Y3 are present in the urine from 0 to 17 years of age.

**Post-translational modifications**

Sulfated.

It is not known whether both or only one of Thr-2064 and Thr-2065 are/is glycosylated.

Forms covalent cross-links mediated by a transglutaminase, such as F13A or TGM2, between a glutamine and the epsilon-amino group of a lysine residue, forming homopolymers and heteropolymers (e.g. fibrinogen-fibronectin, collagen-fibronectin heteropolymers).

Phosphorylated by FAM20C in the extracellular medium.

Proteolytic processing produces the C-terminal NC1 peptide, anastellin.

**Cellular localization**

Secreted, extracellular space, extracellular matrix.

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**Images**

[Western blot image](#)

**All lanes**: Anti-Fibronectin antibody [IST-9] (ab6328) at 1/2000 dilution

**Lane 1**: Human dermal fibroblast whole cell lysate, untreated

**Lane 2**: Human dermal fibroblast whole cell lysate, treated with TGF beta 1

Lysates/proteins at 10 µg per lane.

**Secondary**

All lanes: HRP-conjugated Goat anti-mouse monoclonal at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Exposure time**: 15 seconds

Blocked with 5% milk for 1 hour at 20°C.
**Extracellular matrix expression pattern.**

Fibronectin was displayed in microtissues after ten days. (Bar in A549/SV80 monocultures: 50 μm, bar in all other microtissues: 100 μm); Secretion of fibronectin could be observed in microtissues containing fibroblasts, whereas in all tumour cell monocultures no fibronectin secretion could be detected.

**Correlation of blood vessel diameter and blood vessel coverage with ECM components.**

(PANEL C shown)

(A–D) Blood vessels were visualized by endomucin (green) and coverage of the blood vessel was measured using laminin (red) (A), collagen I (B), fibronectin (C), and collagen I (D). Scale bar: 50 μm.
Anti-Fibronectin antibody [IST-9] (ab6328) at 1/1000 dilution + whole cell lysate prepared from human platelets (treated with ADP for 30 minutes) at 20 µg

**Secondary**

HRP conjugated donkey anti-mouse polyclonal at 1/10000 dilution

Developed using the ECL technique.

**Exposure time:** 2 minutes

Blocked with 5% milk for 1 hour at 22°C.

ab6328 positively staining paraffin fixed canine myocardial infarct tissue (1/200). The images demonstrate the time course of fibronectin deposition in reperfused canine myocardial infarcts.

- **a:** 24 hrs
- **b:** 7 days
- **c:** 28 days of reperfusion.

Secondary: Biotin conjugated goat anti mouse. Detection was achieved using DAB.

This image is an edited version of an image submitted courtesy of an Abreview by Marcin Dobaczewski. We do not have any further information relating to this image.

Formalin fixed paraffin embedded rat kidney stained with ab6328 at a dilution of 1:100 after proteinase K digestion.

The image was kindly supplied as part of the review submitted by Elizabeth Chipala.
ab6328 staining Fibronectin in human small intestine tissue sections by Immunohistochemistry (HC-P - paraformaldehyde-fixed, paraffin-embedded sections).

Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a Tris-EDTA buffer pH 9.0. Samples were incubated with primary antibody (1/50 in blocking buffer) for 30 minutes at 20°C. An undiluted HRP-conjugated Goat anti-mouse polyclonal was used as the secondary antibody.

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