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## **EDB+ FIBRONECTIN FROM WI38VA (SV40 Transformed Fibroblast Cells) PURIFIED BY AFFINITY CHROMATOGRAPHY ON IST-6 SEPHAROSE**

*Fibronectins (FN) are the product of a single gene localized on chromosome 2, but different isoforms arise from the alternative splicing of the pre-mRNA in three sites called IIICS (type III connecting sequence), ED-A (a complete type III repeat, extra domain A) and ED-B (a complete type III repeat, extra domain B). For ED-A and ED-B the usage or skipping of exons leads to inclusion or exclusion of these type III repeats. In cultured transformed cells and in malignancies, the splicing pattern of FN pre-mRNA is altered, leading to an increased expression of FN isoforms containing the domains regulated by alternative splicing. In particular almost 100% of fibronectin molecule from WI38VA cells contain ED-B [1,2]. IST-6 is a murine monoclonal antibody that reacts only with FN molecules lacking the ED-B [3]. FN from WI38VA cells was further purified on IST-6 Sepharose to remove all the FN molecules lacking ED-B.*

### **ORDERING INFORMATION**

**Catalog Number: S-P005 (S-h-100%EDB-WI38VA-fn)**

**Lot Number: XXX**

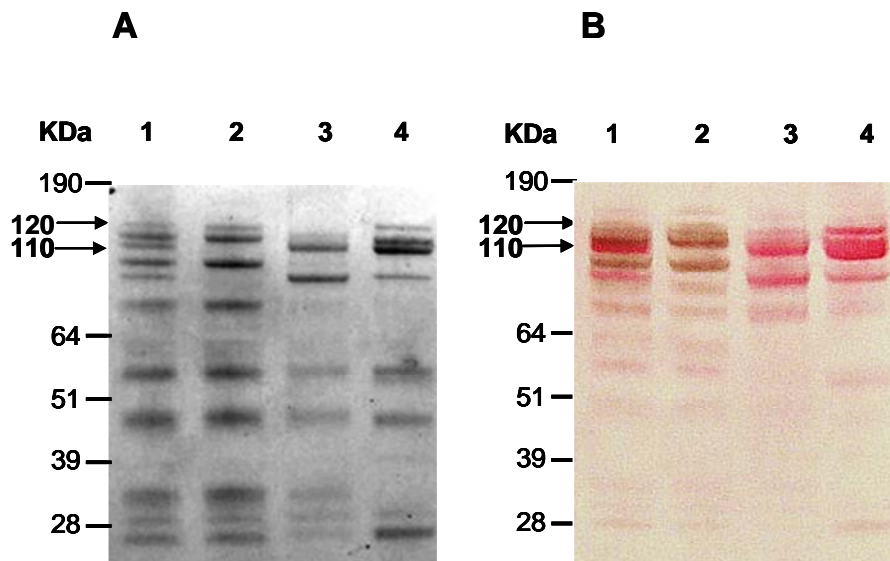
**Size: XX mg/ml; XX ml.**

**Formulation:** in solution in 20 mM sodium phosphate buffer pH=7.6 + 150 mM NaCl. Sterile.

**Storage:** - 20°C. Avoid repeated freeze-thaw cycles.

### **Preparation**

Human fibronectin from WI38VA was purified by affinity chromatography on IST6-Sepharose 4B, specific only for EDB-negative fibronectin.



**Fig.1 (A)** 4-12% SDS-PAGE gradient analysis of FN-WI38VA digested for 4h at 22°C by termolysin (5µg/ml of FN) after purification by immunoaffinity chromatography on IST-6 conjugated to Sepharose-4B. Samples were analyzed under reducing conditions. Lanes: 1) FN-WI38VA not purified on IST-6 column chromatography; 2) 100% EDB FN-WI38VA (unbound to IST-6 column chromatography) 3) FN-WI38VA lacking ED-B (bound to IST-6 column chromatography). 4) human plasma FN as control. The values on the left indicate the molecular masses (KDa). **(B)** Immunoblot analysis of the 4-12 % SDS-PAGE gradient shown in panel A using the monoclonal antibody C-6 (brown) and IST-6 (red). The monoclonal antibody C-6 recognizes the EDB(+) -FN and the monoclonal antibody IST-6 recognizes the EDB(-)-FN .

## References

- [1] Zardi L, Carnemolla B, Siri A, Petersen TE, Paoletta G, Sebastio G, Baralle FE. Transformed human cells produce a new fibronectin isoform by preferential alternative splicing of a previously unobserved exon. *EMBO* 1987;6:2337-42.
- [2] Carnemolla B, Balza E, Siri A, Zardi L, Nicotra MR, Bigotti A, Natali PG. A tumor-associated fibronectin isoform generated by alternative splicing of messenger RNA precursors. *J Cell Biol* 1989;108:1139-48.
- [3] Carnemolla B, Leprini S, Allemanni G, Saginati M, Zardi L. The inclusion of the type III repeat ED-B in the fibronectin molecule generates conformational modifications that unmask a cryptic sequence. *J. Biol. Chem.* 1992; 267:24689-24692;

**PRODUCT DESIGNED FOR RESEARCH USE ONLY AND NOT FOR USE IN HUMANS.**