

Recombinant TFPI-2 Kunitz domain 1 Active

Human recombinant protein expressed in *Nicotiana benthamiana*.

RF007

Molecular formula: C₄₀₇H₅₉₂N₁₁₆O₁₁₇S₆

Molecular weight: 9.3 kDa, 79 amino acids residues

p.I: 6.25

Extinction coefficient : $E^{0.1\%}_{280\text{nm}} = 2.35$ (A_{280nm})

Purity assay: ≥ 97% (SDS-PAGE)

Endotoxin level: < 0.04 EU/ μg protein (LAL method)

Animal-free product*

Sequence:

HHHHHHGAAQEPTGNNAEICLLPLDYGPKALLRYYYDRY
TQSCRQFLYGGCEGNANNFYTWEACDDACWRIEKVPKV

Description: AGV 212 is a protease inhibitor peptide generated from the first Kunitz domain of the human Tissue Factor Protein Inhibitor 2 (TFPI-2) protein, after site-directed mutagenesis to increase its activity. It is arranged in a single polypeptide chain that is linked by three disulfide bridges. AGV 212 is quite stable and inhibits trypsin with high efficiency and K_i lower than TFPI-2 one. TFPI-2 has been shown to inhibit Endothelial Cell Matrix (ECM) proteases essential for angiogenesis and metastasis.

Formulation: Lyophilized powder containing phosphate buffer salts, pH 7.1.

Source: It is produced by transient expression in non-transgenic plants. Recombinant AGV212 includes a 6 His-tag at the N-terminal end, is purified by sequential chromatography (FPLC). This product contains no animal-derived components or impurities.

Reconstitution recommendation: Lyophilized protein should be reconstituted adding 1 ml of sterile water to the vial, which gives a concentration of 1 mg of protease inhibitor per ml. At higher concentration the solubility may be reduced and multimers generated. Soluble in water and in aqueous buffers of low ionic strengths. Repeated freeze-thaw cycles should be avoided.

Storage and Stability: This lyophilized preparation is stable at 2-8° C. For long storage should be kept at -20° C and it is recommended to add a carrier protein (0.1% HSA or BSA). Repeated freezing and thawing is not recommended.

Purity and Serological Identification:

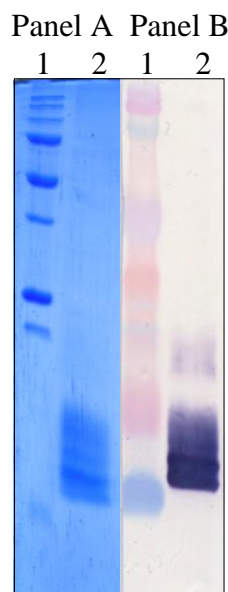


Figure 1. Panel A show SDS-PAGE 15% Coomassie Blue of AGV 212 trypsin inhibitor purified by Gel filtration. Lane 1: Precision Plus Protein Standards cat 161-0363 BIORAD, lane 2: AGV 212 1mg/ml. Panel B show lane 1: Kaleidoscope Prestained Standards cat 161-0324 BIORAD, lane 2: the same samples analyzed by Western Blot with specific antiserum. All bands observed shown the same peptide mass finger printing pattern, corresponding to AGV212. Post-translational modifications do exist among the different bands nevertheless they do not alter their activity

For R+D purposes only. Purchaser must determine the suitability of the product(s) for their particular use.

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Biological Activity: The activity of the inhibitor is expressed as the amount of trypsin inhibited per milligram of inhibitor. The ability to prevent the hydrolysis of benzoyl-L-arginine ethyl ester hydrochloride by trypsin is measured by spectrophotometer.

One mg protein will inhibit 1-1.5 mg trypsin with activity of approximately 10,000 BAEE units per mg protein.

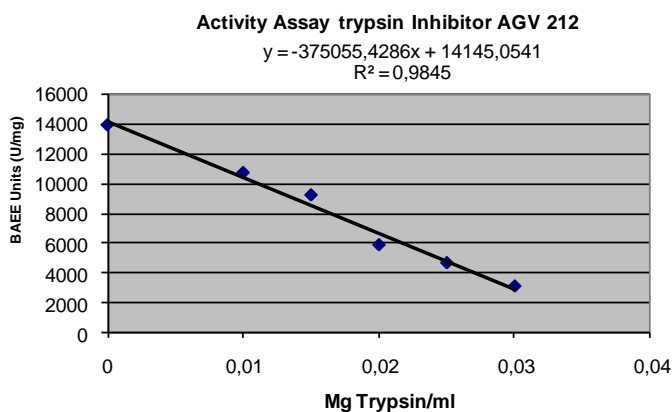


Figure 2. Activity assay of trypsin inhibitor AGV212

References

- Chand, H.S., Schmidt, A.E., Bajaj, S.P. and Kisiel, W. (2004) Structure-function analysis of the reactive site in the first Kunitz-type domain of human tissue factor pathway inhibitor-2. *J. Biol. Chem.* 279, 17500-17507
- Rao, C.N., Lakka, S.S., Kin, Y., Konduri, S.D., Fuller, G.N., Mohanam, S. and Rao, J.S. (2001) Expression of tissue factor pathway inhibitor 2 inversely correlates during the progression of human gliomas. *Clin. Cancer Res.* 7, 570-576
- Konduri, S.D., Tasiou, A, Chandrasekar, N. and Rao, J.S. (2001) Overexpression of tissue factor pathway inhibitor-2 (TFPI-2), decreases the invasiveness of prostate cancer cells in vitro. *Int. J. Oncol.* 18, 127-131.
- Chand, H.S., Du, X., Ma, D., Inzunza, H.D., Kamei, S., Foster, D., Brodie, S. and Kisiel, W. (2004) The effect of human tissue factor pathway inhibitor-2 on the growth and metastasis of fibrosarcoma tumors in athymic mice. *Blood*, 103, 1069-1077.
- Kondraganti, S., Gondi, C.S., Gujrati, M., McCutcheon, I., Dinh, D.H., Rao, J.S. and Olivero, W.C. (2006) Restoration of tissue factor pathway inhibitor inhibits invasion and tumor growth in vitro and in vivo in a malignant meningioma cell line. *Int. J. Oncol.* 29, 25-32.