TFPI-2 Kunitz domain 1, active, human recombinant protein, expressed in Nicotiana benthamiana, His Tag, animal free

AGV 212 Trypsin Inhibitor

Catalog No: 99858

Lot No:

Source: Nicotiana benthamiana

Molecular formula: $C_{407}H_{592}N_{116}O_{117}S_6$

Molecular weight: 9.3 kDa, 79 amino acids residues

p.I: 6.25

Extinction coefficient: E 0.1% = 2.35 (A 280 nm)

Purity assay: ≥ 97% (SDS-PAGE)

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

Sequence:

HHHHHHGAAQ EPTGNNAEIC LLPLDYGPCK ALLLRYYYDR YTQSCRQFLY GGCEGNANNF YTWEACDDAC WRIEKVPKV

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 Ki 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, but should be kept at –20°C for long term storage. Diluted solutions are less stable than concentrated ones. Repeated freezing and thawing is not recommended.

Purity and Serological Confirmation:

Panel A Panel B

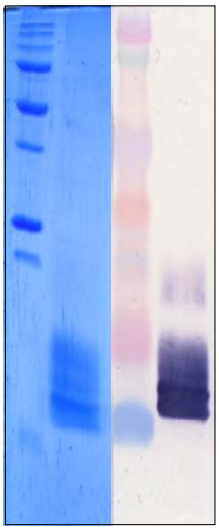

Figure 1.
Panel A show SDS-PAGE 15% Coomassie Blue of AGV 212 trypsin inhibitor purified by Gel filtration.
Lane 1: Precision Plus Protein Standards cat 161-0363 BIORAD
Lane 2: AGV 212 1mg/ml,.
Panel B show
Lane 1: Kaleidoscope Prestained Standards cat 161-0324 BIORAD
Lane 2: the same samples analyzed 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

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.

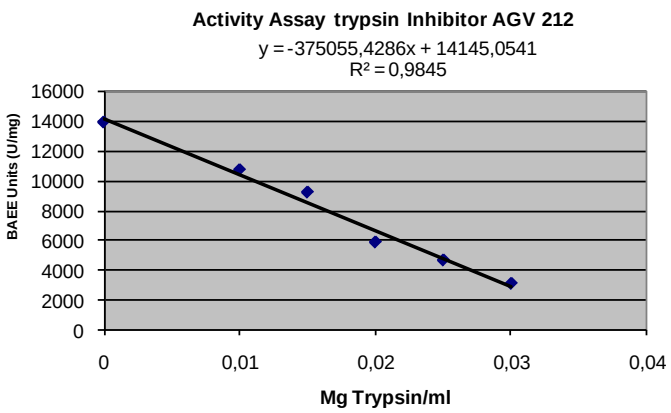

Figure 2. Activity assay of trypsin inhibitor AGV212 by procedure Sigma Aldrich # 10-30-6920, (ability to prevent the hydrolysis of benzoyl-L-arginine ethyl ester hydrochloride by trypsin) 1 mg protein will inhibit 1-1.5 mg trypsin with activity of approximately 10,000 BAEE units per mg protein. (one trypsin unit = ΔA253 of 0.001 per min with BAEE as substrate at pH 7.6 at 25 ºC. reaction volume = 3.2 ml (1 cm light path)

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.