Product Data Sheet

Product Name:	WAAG-3R, Aggrecanase (ADAM-TS-4) FRET Substrate
Catalog Number:	AS-60431-1 (1 mg) Lot Number: See label on vial
Sequence:	Abz-Thr-Glu-Gly-Glu-Ala-Arg-Gly-Ser-Val-Ile-Dap(Dnp)-Lys-Lys-NH2 (3-letter code) Abz-TEGEARGSVI-Dap(Dnp)-KK-NH2 (1-letter code)
Molecular Weight:	1645.8
% Peak Area by HPLC:	≥ 95
Appearance:	Lyophilized yellow powder

Peptide Reconstitution: WAAG-3R peptide is freely soluble in H_2O.

Storage: WAAG-3R peptide is shipped at ambient temperature. Upon receipt, store lyophilized peptide at –20°C or lower. Reconstituted peptide can be aliquoted and stored at –20°C or lower.

Description: This FRET peptide was used in an ADAM-TS-4 (Aggrecanase-1) assay. Ex/Em = 340/420 nm. Ref: Zhang, Y. et al. *J. Pharmacol. Exp. Ther.* **309**, 348 (2004).

Additional Information: *Listed below are relevant information that may provide a guideline on how to use this product. End users will have to adapt to their own specific applications.*

The ADAM-TS-4 (Aggrecanase-1) assay was performed using a fluorescent peptide Abz-TEGEARGSVI-Dap(Dnp)-KK (denoted as WAAG-3R, custom synthesized by AnaSpec, Inc.). The assay buffer contains 50 mM HEPES, pH 7.5, 100 mM NaCl, 5 mM $CaCl_2$, 0.1% CHAPS, and 5% glycerol. Total reaction volume is 100 µl. The recombinant Agg-1 proteins generated at Wyeth Research (final concentration of 5 µg/ml in the assay) were pretreated with the various concentrations of the compound for 10 to 15 min at 37°C. The reaction was initiated by addition of the WAAG-3R substrate at a final concentration of 25 µM- Zhang, Y. et al. *J. Pharmacol. Exp. Ther.* **309**, 348 (2004).

The peptide sequence was Abz-TEGEARGSVI-Dap(Dnp)-KK (Anaspec) and derived from the aggrecanase cleavage site of aggrecan. The final concentration of substrate in the assay was µ25 M. The buffer used in this assay was 50 mM HEPES (pH 7.5), 100 mM NaCl, 5 mM $CaCl_2$, 0.1% CHAPS, and 5% glycerol. Compounds (in duplicate) were serially diluted from 2 mM to 0.01 µM in 100% DMSO. The total reaction volume was 100 µL. The buffer and enzyme were added first followed by addition of 10× inhibitor. Enzyme and buffer alone samples were included in order to obtain the maximal rate of substrate cleavage. The reaction was allowed to stand for 15 min at 30°C- Mosyak, L. et al. *Protein Science* **17**, 16 (2008).

Published Citations:

Zhang, Y. et al. *J. Pharmacol. Exp. Ther.* **309**, 348 (2004).
Mosyak, L. et al. *Protein Science* **17**, 16 (2008).

Related Products:

Name	Cat #	Size
SensoLyte® 520 Aggrecanase-1 Assay Kit *Fluorimetric*	AS-72114	1 kit

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