PRODUCT DATA SHEET
ANTI-HUMAN RAGE C2 DOMAIN MONOCLONAL ANTIBODY

PRODUCT INFORMATION

Catalog Number:	GM-1303	**Clone:**	B0077-C03-D11
Description:	purified monoclonal mouse antibody	**Specificity:**	anti-human RAGE C2 domain
Isotype:	IgG1/kappa	**Purification:**	Protein G
Storage:	short term: 2– 8°C; long term: –80°C (avoid repeated freeze / thaw cycles)	**Buffer:**	phosphate buffered saline, pH 7.4
Immunogen:	genetic immunization with cDNA encoding human RAGE	**Concentration:**	1.0 mg/mL
Tested Applications	Western blot, ELISA, SPR		

WORKING DILUTIONS

Flow Cytometry:	5 µg/10^6 cells		
ELISA:	1:250 – 1:1000	**Western Blot:**	1:500 – 1:2000
For each application, a titration should be performed to determine the optimal concentration.			

ANALYTICAL SEC OF B0077-C03-D11

The antibody was purified by protein G affinity chromatography from clarified cell culture supernatant. Purity was tested using analytical size exclusion chromatography (Fig. 1).

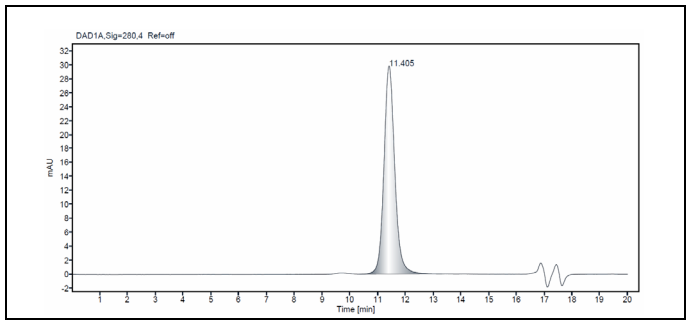

Fig. 1: Analytical SEC of the purified antibody. Conditions: Tosoh TSKgel G3000SWxl (7.8 x 30 cm + guard column), 20 mM Sodium Phosphate, 0.3 M L-Arginine, pH 7.8 mobile phase, 0.75 mL/min, 22°C, 280 nm detection. Purity is >95%.

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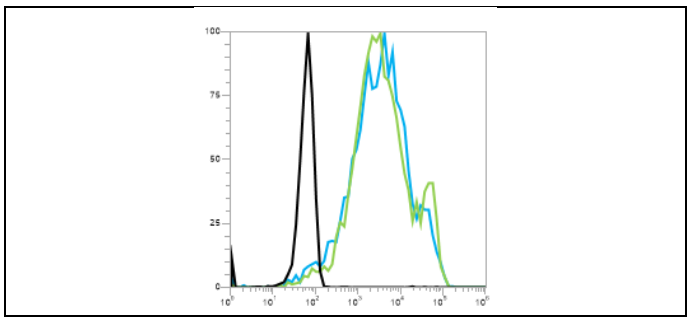

Fig. 2: FACS. BOSC23 cells were transiently transfected with an expression vector encoding human RAGE or human es RAGE. Binding was detected with a PE conjugated secondary antibody. Blue: cells transiently transfected with human RAGE; green curve: human cells transiently transfected with esRAGE; black: non-transfected cells.

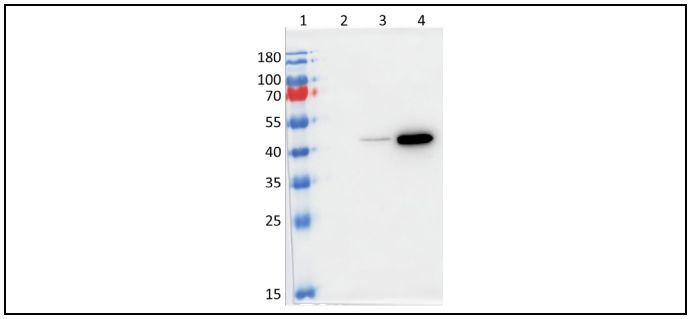

Fig. 3: Western blot. Purified RAGE extracellular domain was separated by SDS-PAGE and transferred to a nitrocellulose membrane. Following blocking with TBS + 5% nonfat dried milk + 0.5% Tween-20, blots were incubated with a 0.5 µg/mL antibody, washed, and then incubated with an HRP-conjugated secondary antibody. Bands were detected using a chemiluminescent substrate. Lane 1: MW marker; lane 2: 1 ng RAGE ECD; lane 3: 10 ng RAGE ECD; lane 4: 100 ng RAGE ECD

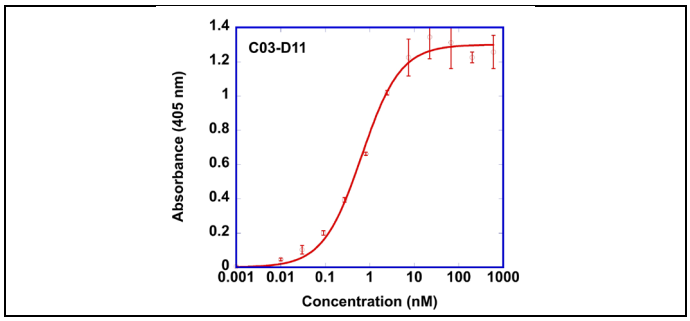

Fig. 4: ELISA. High-binding ELISA plates were coated with 20 µg/mL of purified RAGE extracellular domain and then blocked with PBS + BSA. Serial dilutions of the antibody were incubated for 1 hour at room temperature and then washed. Binding was detected using an HRP-conjugated secondary antibody and a PNPP substrate. Data was fit using a 1:1 Langmuir binding model.

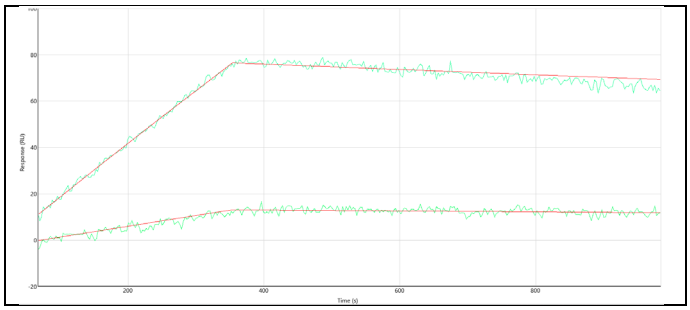

Fig. 5: SPR. Binding to the purified RAGE extracellular domain was measured using the Carterra LSA. Antibody was printed on an Protein A/G HC30M chip, and binding measured for two concentrations of purified RAGE extracellular domain. The measured K_D value was 0.3 nM.

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BACKGROUND

The Receptor for Advanced Glycation End Products (RAGE, UniProt ID Q15109) is a type I integral membrane protein that binds advanced glycosylation end products (AGE). The 50–55 kDa glycosylated protein contains an extracellular domain (aa 23–342), a hydrophobic transmembrane domain (aa 343–363), and a cytoplasmic domain (aa 363–404). The RAGE extracellular region is composed of a variable (V) immunoglobulin domain (aa 23–116) and two constant Ig domains (C1, aa 124–221; C2, aa 227–317) connected by a flexible seven amino acid linker. RAGE is associated with several diseases, including diabetic complications, Alzheimer's disease, cancer, Lupus, and hypertension.

Antibody B0077-C03-D11 binds specifically to the C2 domain of the human RAGE protein, demonstrated using a dot-blot screen and an ELISA against purified V, C1 and C2 domains of RAGE. The antibody binds to purified RAGE extracellular domain with K_D = 0.3 nM, measured using high throughput SPR (Figure 5).

REFERENCES

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