



Validation and Characterization of Anti-Citrulline Antibodies and Citrullinated Proteins

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KEY FINDING SPR confirms high-affinity, specific recognition of citrullinated proteins, supporting reliable detection of protein citrullination.

INTRODUCTION

Citrulline is a non-proteinogenic amino acid that is produced by deimination of arginine through the post-translational modification known as citrullination.¹ Citrullination is catalyzed by protein arginine deiminases (PADs) which convert positively charged arginine to electrically neutral citrulline, decrease the isoelectric point of the protein, alter the native protein structure, and affect protein ionic interactions.² Protein citrullination plays a vital role in many physiological and pathological processes including autoimmunity, cancer, and neurodegenerative disorders.³ Here we validate and characterize the anti-citrulline antibodies produced by Cayman through Immunofluorescence, ELISA, and Surface Plasmon Resonance.

RESULTS

To validate citrullination, Cayman's Anti-Citrulline Monoclonal Antibody (Clone 1D9) (Item No. 30773) was used for Immunofluorescence and ELISA. Surface Plasmon Resonance (SPR) was subsequently employed to characterize the binding kinetics.

Neutrophil Extracellular Traps Immunofluorescence

Primary neutrophils stimulated with 100 nM PMA to induce neutrophil extracellular traps (NETs) formation were fixed with 3.7% PFA and blocked with 5% normal goat serum. Cells were probed with a mouse IgG1 isotype control **(A)** or the Anti-Citrulline Monoclonal Antibody (Clone 1D9) (Item No. 30773) at a 1:200 dilution **(B)** followed by secondary antibody Goat Anti-Mouse (IgG+IgM) FITC (Item No. 10006617). Cell nuclei were stained with DAPI.

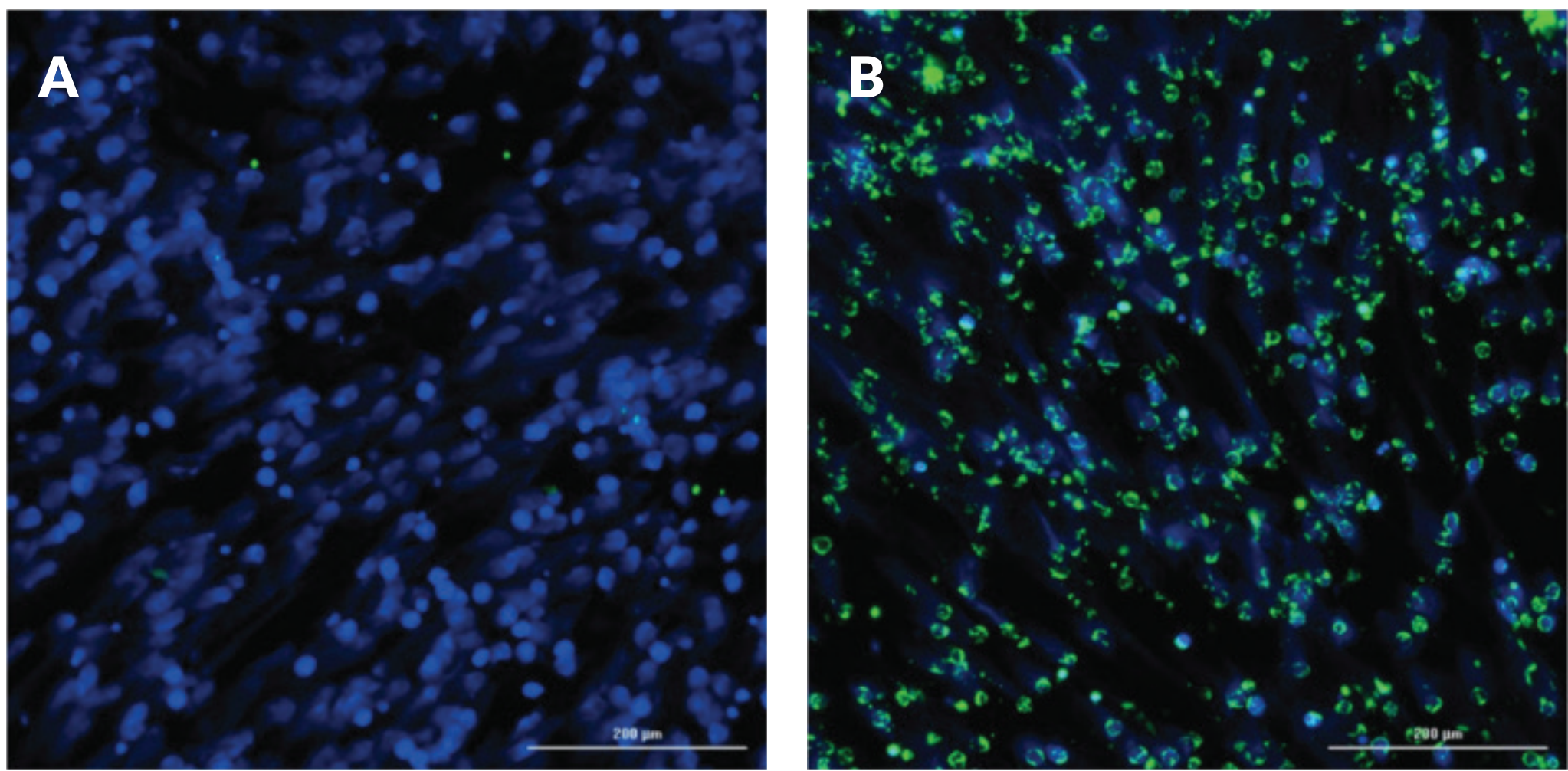


FIGURE 1 – Validation of histone citrullination by IF.

ELISA Validation

Proteins were coated at 1 μg/ml in PBS and blocked with 1% BSA. Plates were probed with serially diluted anti-citrulline monoclonal antibody (Item No. 30773) starting at either 1 or 2 μg/ml, followed by a goat anti-mouse IgG HRP-conjugated secondary antibody (Item No. 10004302). Signal was developed using TMB, stopped with H₂SO₄, and absorbance was measured at 450 nm.

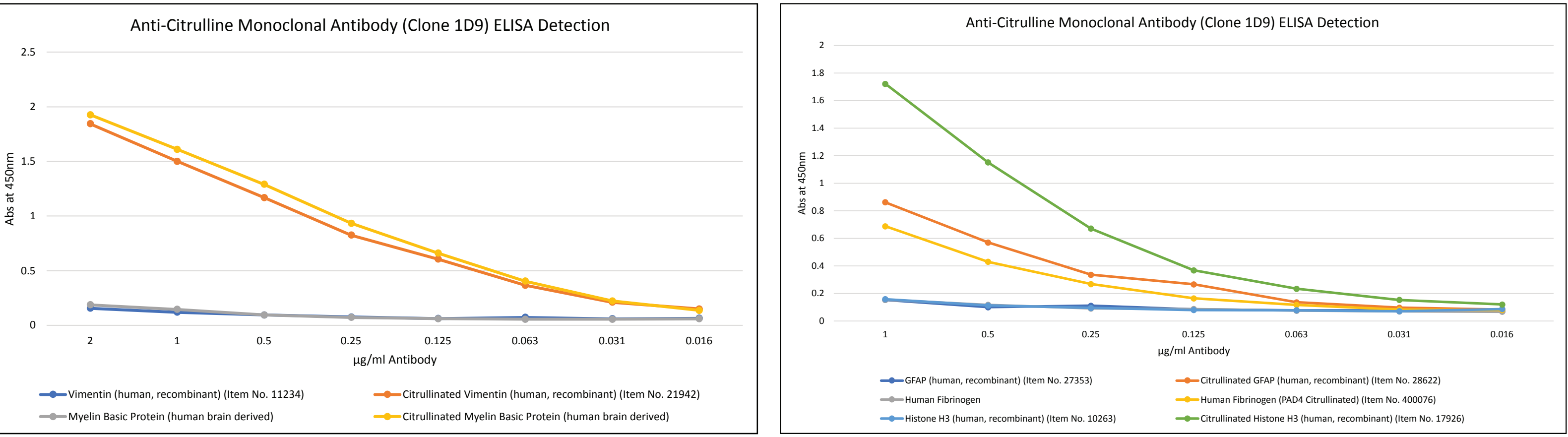


FIGURE 2 – Validation on protein citrullination by ELISA.

Characterization of Anti-Citrulline Antibodies by SPR

SPR experiments were performed on a Biacore™ 8K instrument. Antibodies were immobilized to a Series S Sensor Chip CM5 (Cytiva) through amine coupling (5,000-7,000 RU). The antigens diluted in 1xHBS-EP+ (Cytiva) were injected at concentrations ranging from 2.4 nM to 1.5 μM at a flow rate of 30 μl/min and at a temperature of 25°C. Association and dissociation were carried out for 120 s and 600 s, respectively, with regeneration steps included between cycles. The data were fit to a 1:1 binding model with Biacore Evaluation software.

RESULTS (CONTINUED)

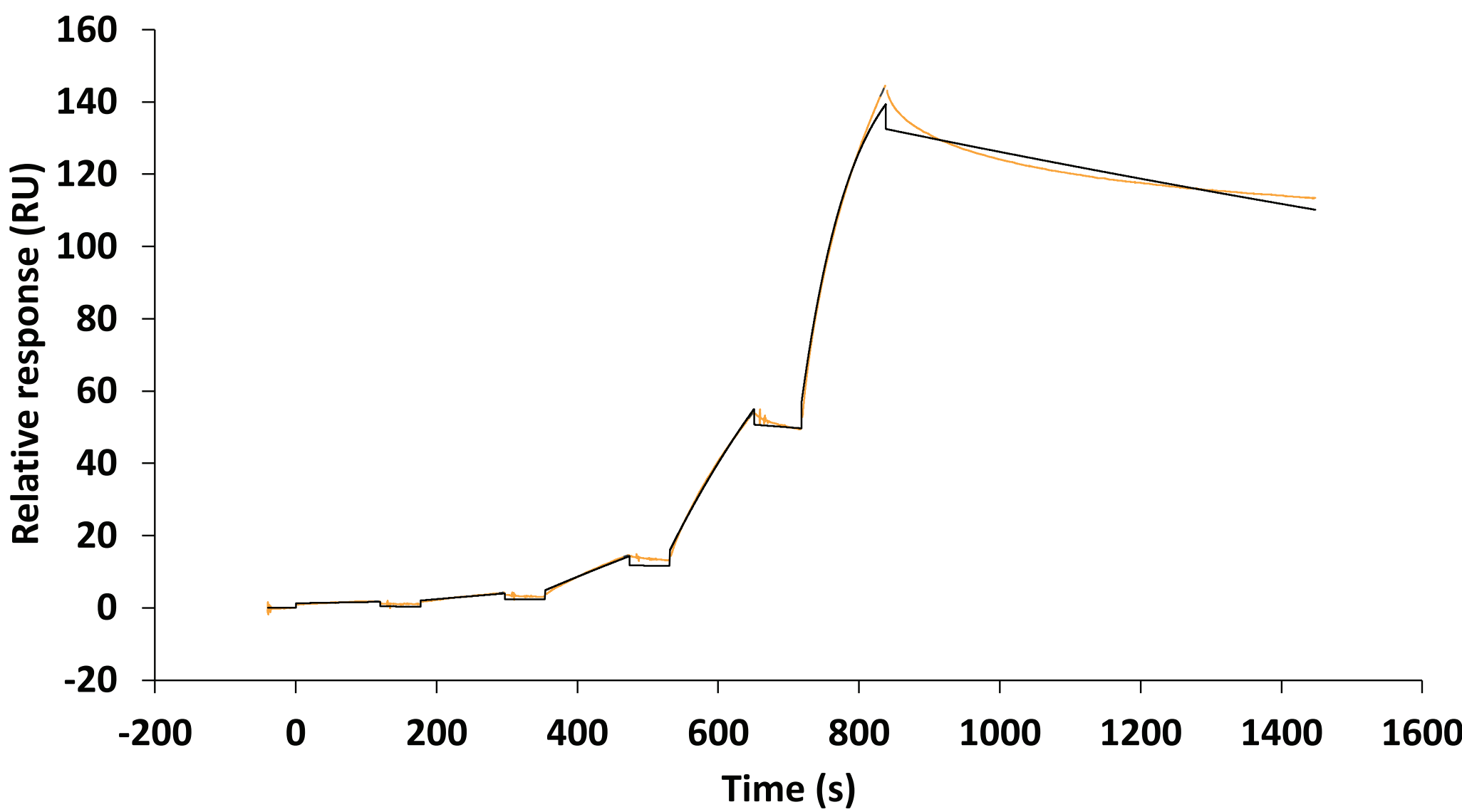


FIGURE 3 – Single Cycle Kinetics (SCK) Sensorgrams. Citrullinated Vimentin against Citrullinated Vimentin mAb.

TABLE 1 – 1:1 Binding Kinetics for Citrullinated Vimentin and Fibrinogen Antibodies.

Analyte	Ligand	k _a (1/Ms)	k _d (1/s)	K _D (μM)	Rmax (RU)
Citrullinated Vimentin (human, recombinant) (Item No. 21942)	Citrullinated Vimentin Monoclonal Antibody (Clone 12G11) (Item No. 22054)	9.15E+03	3.03E-04	3.31E-08	154.8
Human Fibrinogen (PAD4 Citrullinated) (Item No. 400076)	Citrullinated Fibrinogen Monoclonal Antibody (Clone 10E9.3) (Item No. 17088)	3.20E+04	2.85E-03	8.89E-08	405.8

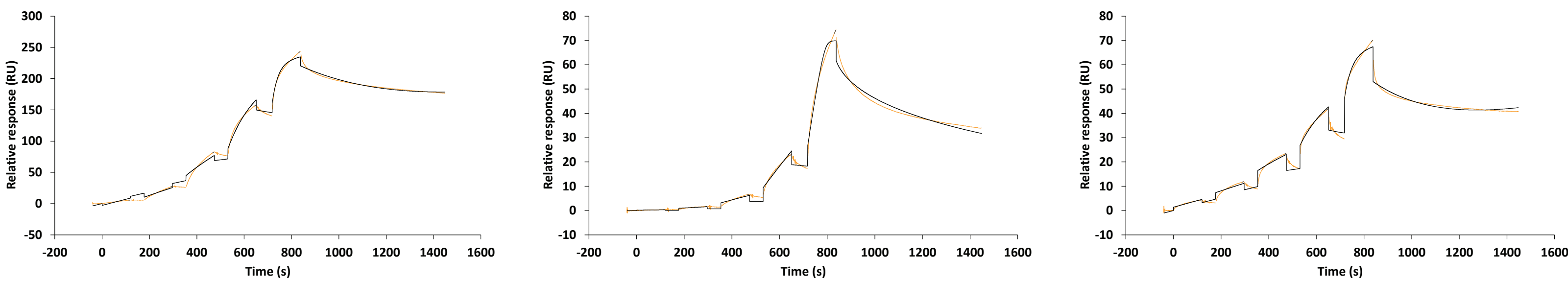


FIGURE 3 – Single Cycle Kinetics (SCK) Sensorgrams. SCK Sensorgram for citrullinated proteins against Anti-Citrulline mAb (Left) Citrullinated Vimentin (Center) Citrullinated Fibrinogen (Right) Citrullinated GFAP.

TABLE 2 – 1:1 Binding Kinetics for Anti-Citrulline Antibody

Analyte	Ligand	k _a (1/Ms)	k _d (1/s)	K _D (μM)	Rmax (RU)
Citrullinated Vimentin (human, recombinant) (Item No. 21942)	Anti-Citrulline Monoclonal Antibody (Clone 1D9) (Item No. 30773)	1.52E+04	1.24E-03	1.07E-07	103.3
Human Fibrinogen (PAD4 Citrullinated) (Item No. 400076)	Anti-Citrulline Monoclonal Antibody (Clone 1D9) (Item No. 30773)	1.38E+05	7.12E-03	5.15E-08	63.8
Citrullinated GFAP (human, recombinant) (Item No. 28622)	Anti-Citrulline Monoclonal Antibody (Clone 1D9) (Item No. 30773)	1.93E+04	2.97E-03	1.54E-07	35.2

CONCLUSIONS

- Citrullination was validated by IF and ELISA.
- SPR binding analysis validated the interaction of citrullinated proteins with Anti-Citrulline antibodies and specific citrullinated protein antibodies.



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