Monolith Protocol MO-P-040

Protein A – Rabbit & Mouse IgG

Protein A is a cell wall component produced by several strains of *Staphylococcus aureus*. The protein A molecule contains four high affinity binding sites capable of interacting with the Fc region of IgG from several species, including rabbit. Conversely, protein A has a much lower affinity towards mouse IgGs. Given the high affinity of protein A to numerous IgGs, it is commonly used in biochemical research for immunoassays such as Western blotting, immunohistochemistry and ELISA.

protein – protein interaction | antibody

A1. Target/Fluorescent Molecule

Protein A
uniprot.org/uniprot/P38507

A2. Molecule Class/Organism

Immunoglobulin-binding protein
Staphylococcus aureus

A3. Sequence/Formula

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MKKKNIYSIR KLGVGIASVT LGTLLISGGV TPAANAAQHD EAQQNAFYQV LNMPNLNADQ RNGFIQSLKD DPSQSANVLG
EAQKLNDSQA PKADAQQNKF NKDQQSAFYE ILNMPNLNEE QRNGFIQSLK DDPSQSTNVL GEAKKLNESQ APKADNNFNK
EQQNAFYEIL NMPNLNEEQR NGFIQSLKDD PSQSANLLAE AKKLNESQAP KADNKFNKEQ QNAFYEILHL PNLNEEQRNG
FIQSLKDDPS QSANLLAEAK KLNDAQAPKA DNKFNKEQQN AFYEILHLPN LTEEQRNGFI QSLKDDPSVS KEILAEAKKL
NDAQAPKEED NNKPGKEDGN KPGKEDGNKP GKEDNKKPGK EDGNKPGKED NKKPGKEDGN KPGKEDGNKP GKEDGNKPGK
EDGNKPGKED GNGVHVVKPG DTVNDIAKAN GTTADKIAAD NKLADKNMIK PGQELVVDKK QPANHADANK AQALPETGEE
NPFIGTTVFG GLSLALGAAL LAGRRREL
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A4. Purification Strategy/Source

Recombinant
Thermo Scientific™ Pierce™
PI21184

A5. Stock Concentration/Stock Buffer

5 mg/mL | 111 µM
Phosphate-buffered saline (PBS, pH 7.4)

A6. Molecular Weight/Extinction Coefficient

45 kDa
8,940 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

Phosphate-buffered saline (PBS, pH 7.4), 0.05% TWEEN® 20, 0.4 mg/mL BSA[1]

A8. Labeling Strategy

Monolith Protein Labeling Kit RED – NHS 2nd Generation (MO-L011, NanoTemper Technologies GmbH)
1* Labeling Buffer NHS | 1* Dye RED-NHS 2nd Generation (10 µg) | 1* B-Column

A9. Labeling Procedure

1. Add 82 µL of PBS to 18 µL of 111 µM protein A to obtain 100 µL of a 20 µM solution.
2. Add 25 µL of DMSO to Dye RED-NHS 2nd Generation (10 µg) to obtain a ~600 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
3. Mix 10 µL of the 600 µM dye solution with 90 µL of Labeling Buffer NHS to obtain 100 µL of a 60 µM dye solution (3x protein concentration).
4. Mix protein A and dye in a 1:1 volume ratio (200 µL final volume, 5% final DMSO concentration).
5. Incubate for 30 minutes at room temperature in the dark.
6. In the meantime, remove the top cap of the B-Column and pour off the storage solution. Remove the bottom cap and place with adapter in a 15 mL tube.
7. Fill the column with dilution buffer and allow it to enter the packed resin bed completely by gravity flow. Discard the flow through collected. Repeat this step 3 more times.
8. Add 200 µL of the labeling reaction from step 4 to the center of the column and let sample enter the bed completely.
9. Add 300 µL of dilution buffer after the sample has entered and discard the flow through.
10. Place column in a new collection tube, add 600 µL of dilution buffer and collect the eluate.
11. Hard spin labeled protein A at 21,000 × g for 10 minutes at 4°C. Carefully remove supernatant and re-tube.
12. Keep the labeled protein A (~3 µM) on ice in the dark.

A10. Labeling Efficiency

Measurement of protein concentration and degree of labeling (DOL) using a NanoDrop™:
nanotempertech.com/dol-calculator

Absorbance A_{205}	4.41	Protein concentration	3.0 µM
Absorbance A_{650}	1.14	Degree-of-labeling (DOL)	1.95

[1] BSA was added to labeled protein A only after elution from column B so that samples could be tested on Tycho for quality assessment and NanoDrop for concentration quantification.

B1. Ligand/Non-Fluorescent Binding Partner

Rabbit IgG
Mouse IgG

B2. Molecule Class/Organism

Rabbit (*Oryctolagus cuniculus*) Polyclonal Antibodies
Mouse (*Mus musculus*) Monoclonal Antibodies

B3. Sequence/Formula

N/A

B4. Purification Strategy/Source

Rabbit IgG	Mouse IgG_1 (3E8)
Thermo Fisher Scientific	Santa Cruz Biotechnology Inc.
31235	sc-69786

B5. Stock Concentration/Stock Buffer

Rabbit IgG	Mouse IgG_1
11.4 mg/mL | 76 µM	100 µg/mL | 667 nM
PBS, pH 7.6	PBS, 1% glycerol, 0.1% gelatin, <0.1% sodium azide, <0.1% stabilizer protein

B6. Molecular Weight/Extinction Coefficient

150 kDa
~210,000 $M^{-1}cm^{-1}$ (ε_{280})

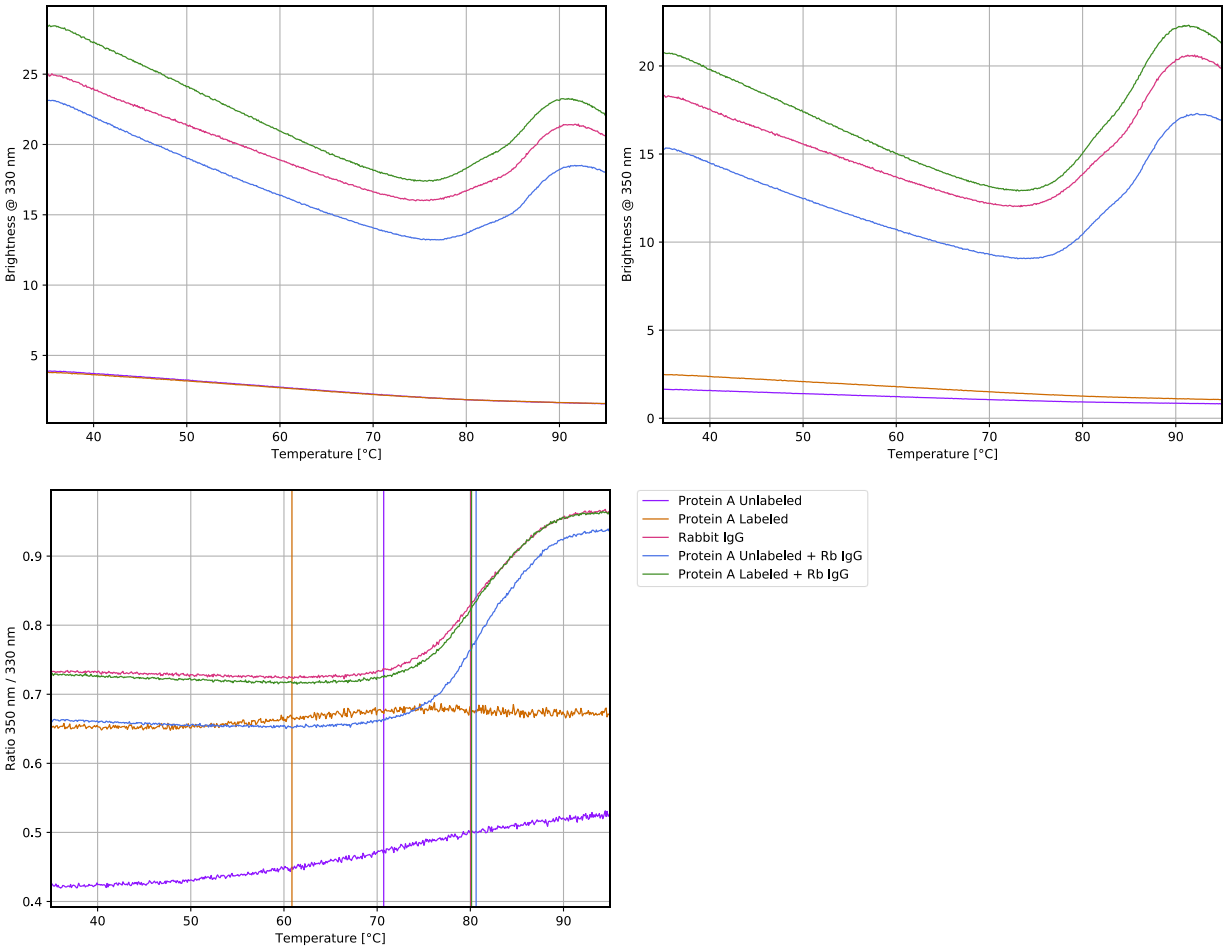
B7. Serial Dilution Preparation

1. Prepare a 100 nM solution of rabbit IgG by performing a multi-step dilution in dilution buffer. First perform a 100X dilution by transferring 1 µl of Rabbit IgG into 99 µl of dilution buffer. Next, transfer 3.3 µl of this solution into 21.7 µl of dilution buffer.
2. Prepare a 100 nM solution of mouse IgG by transferring 3.7 µl of the stock mouse IgG solution into 22.3 µl of dilution buffer.
3. Prepare a 1:1 serial dilution of the two ligands in a 384-well low bind MTP plate using 24 wells for each K_d to be measured. Transfer 20 µL of the 100 nM IgG solutions into well column 1. Then, transfer 10 µL of dilution buffer into tubes **2** to **24**.
4. Prepare a 1:1 serial dilution by transferring 10 µL well to well. Mix carefully by pipetting up and down. Remember to discard 10 µL from well **24** to get an equal volume of 10 µL for all samples.
5. Dilute protein A 100X by mixing 1 µl of labeled protein with 99 µl of dilution buffer to obtain 30 nM.
6. Mix 5 µL of 30 nM labeled protein A with 295 µL of dilution buffer to obtain 300 µL of ~500 pM protein A.
7. Add 10 µL of 500 pM labeled protein A to each well from **24** to **1** and mix by pipetting.
8. Incubate for 30 minutes at room temperature in the dark before loading capillaries.

C. Applied Quality Checks

Validation of structural integrity and functionality of labeled protein A using Tycho NT.6[2]:
nanotempertech.com/tycho

Protein A (UL)	3.3 µL of 11.1 µM protein A + 9.2 µL of dilution buffer	T_i = 70.7°C
Protein A (L)	10 µL of Column B eluate (~3 µM)	T_i = 60.9°C
Rabbit IgG (UL)	1 µL of 76 µM rabbit IgG + 24 µL of dilution buffer	T_i = 80.0°C
Protein A (UL) + Rabbit IgG	3.3 ul of 11.1 µM protein A + 1 ul of 76 µM rabbit IgG + 20.7 µl dilution buffer	T_i = 80.6°C
Protein A (L) + Rabbit IgG	24 µl of B-Column eluate + 1 µl of 76 µM rabbit IgG	T_i = 80.1°C



[2] TSA was not tested with mouse IgG due to the low stock concentration.

D1. MST System/Capillaries

Monolith NT.Automated Pico-Red (NanoTemper Technologies GmbH)
Premium Capillary Chips Monolith NT.Automated (MO-AK005, NanoTemper Technologies GmbH)

D2. MST Software

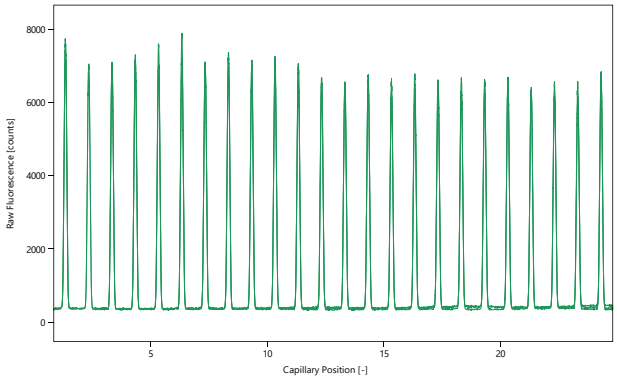
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

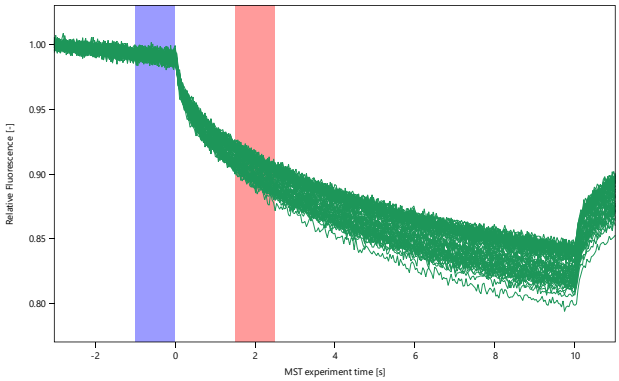
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

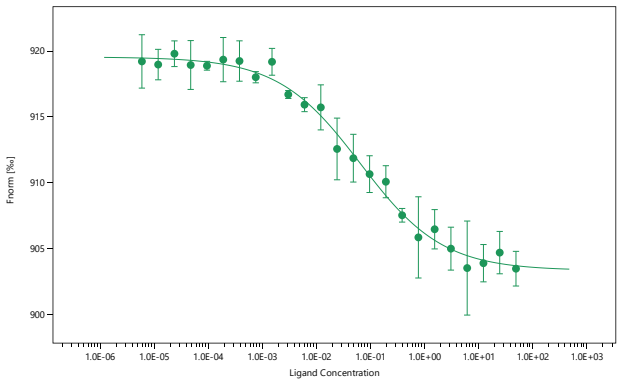
Phosphate-buffered saline (PBS, pH 7.4), 0.05% TWEEN® 20, 0.4 mg/mL BSA
250 pM protein A | 50 nM – 5.96 fM IgG | 25°C | medium MST power | 60% excitation power

D4. MST Results (Capillary Scan/Time Traces/Dose Response)

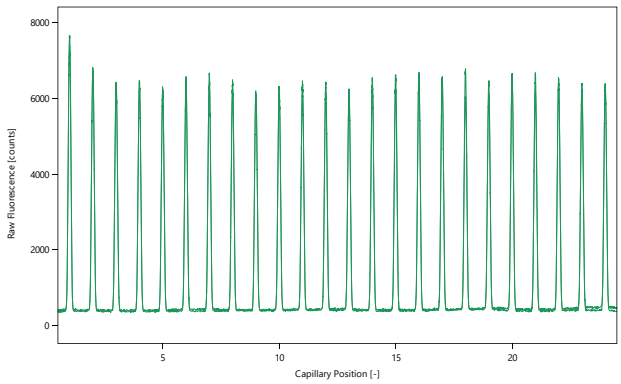
EC_{50} = 66 ± 13 pM (Rabbit IgG)

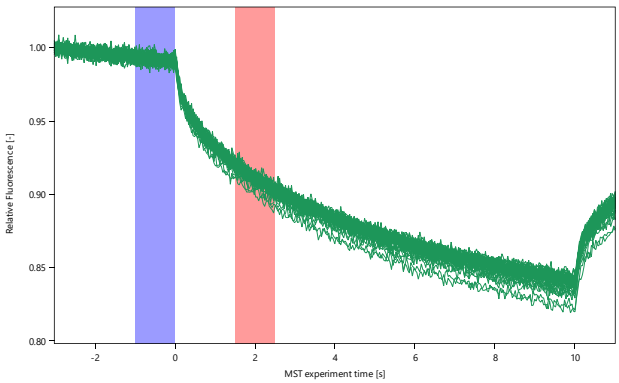


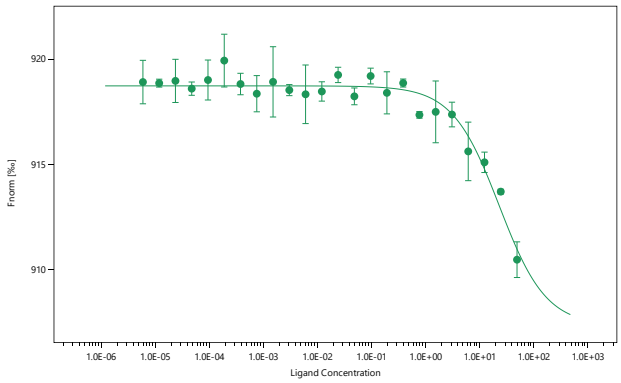




K_d = 23 ± 8 nM (Mouse IgG, binding curve does not reach saturation)







D5. Reference Results/Supporting Results

Protein A + Rabbit IgG Interaction = ++++,
Protein A + Mouse IgG_1 Interaction = +

Competitive ELISA[3]
GE Healthcare, Affinity Chromatography Vol. 1 Antibodies 18103746

E. Contributors

Brett Thurlow[4]

[3] No measurement of K_ds.
[4] NanoTemper Technologies Inc., San Francisco, CA, USA | nanotempertech.com