Monolith Protocol MO-P-079

# Antibody – TNT

The illegal use of explosives by terrorists and other criminals is an increasing issue in public spaces, such as airports, railway stations, highways, sports venues, theaters, and other large buildings. The fast and extremely sensitive detection of explosives is one of the most relevant tasks to guarantee security in areas of public access. 2,4,6-trinitrotoluene (TNT) is a small chemical compound, which is best known as an explosive material with convenient handling properties. In this study the binding between an antibody and TNT was analyzed.

protein – small molecule interaction | TNT | Explosives

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## A1. Target/Fluorescent Molecule

Monoclonal antibody Clone A.1.1.1

## A2. Molecule Class/Organism

Immunoglobulin IgG1

mouse

## A3. Sequence/Formula

N/A

## A4. Purification Strategy/Source/Batch-No.

Hydrophobic Interaction Chromatography | SDIX (Newark,USA) | 107415

## A5. Stock Concentration/Stock Buffer

10.9 g/L | 73 µM

PBS with 0.05 % $NaN_3$,

## A6. Molecular Weight/Extinction Coefficient

150 kDa

## A7. Assay Buffer

8.1 mM disodium phosphate, 1.5 mM potassium dihydrogen phosphate, 2.7 mM potassium chloride, 137 mM sodium chloride, pH 7.4, 0.1% BSA

## A8. Labeling Strategy

Fluorescent labeling of the monoclonal antibody A.1.1.1 for MST measurements was done using the Dy-654-NHS labeling kit (#654-01) according to the manufacturer's protocol (Dyomics GmbH, Jena, Germany).

## A9. Labeling Procedure

1. Prepare 100 mL coupling buffer containing 137 mM NaCl and 100 mM phosphate (pH 7.8).
2. Prepare 100 mL PBS (8.1 mM disodium phosphate, 1.5 mM potassium dihydrogen phosphate, 2.7 mM potassium chloride, 137 mM sodium chloride, pH 7.4)
3. Prepare 100 µL of a 13 µM A.1.1.1 solution in PBS.
4. Dissolve 200 µg of Dy-654-NHS in 40 µL of dry amine-free DMF to obtain a 4.53 mM solution (5g/L).
5. Equilibrate a SpinTrap G-25 column (Cytiva) four times with140 µL coupling buffer and centrifuge at 800 g for one minute and 4°C.
6. Add the 100 µl A.1.1.1 solution (2 g/L, 13 µM) to the SpinTrap, subsequently add 40 µl of coupling buffer, centrifuge and collect the 140 µL eluate.
7. Add 1.68 µL of the 4.53 mM Dy-654-NHS solution (7.6 nmol) to the 140 µl antibody solution and mix immediately by careful resuspending with the pipette. Incubate for two hours at RT in a shaker at 800 rpm.
8. Equilibrate a SpinTrap four times with 140 µL of PBS as described above. Add the labeling reaction on the SpinTrap and centrifuge at 800 g for one minute at 4°C. As eluate 140 µL of light blue solution of approx. 10 µM A1.1.1-Dy654 are obtained.
9. Mix 1.5 µl of the labeled A.1.1.1 with 7 498 µL assay buffer to obtain 7 500 mL of ~2 nM A.1.1.1-Dy-654

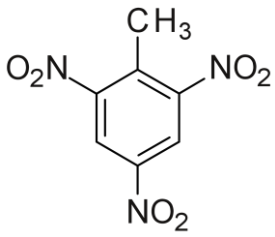
## B1. Ligand/Non-Fluorescent Binding Partner

2,4,6-trinitrotoluene (TNT)

## B2. Molecule Class/Organism

Small molecule (explosives)

## B3. Sequence/Formula




$C_6H_2(NO_2)_3CH_3$

## B4. Purification Strategy/Source/Batch-No.

Supplied by BAM division 2.3

## B5. Stock Concentration/Stock Buffer

4.54 g/L | 20 mM
Absolute Ethanol

## B6. Molecular Weight

227 g/mol

## B7. Serial Dilution Preparation

1. Dilute 5 µL of 20 mM TNT stock in 995 µL absolute ethanol to obtain a 100 µM TNT solution
2. Dilute 2 µL of 100 µM TNT solution with 398 µL assay buffer to obtain a 500 nM TNT solution
3. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 500 nM TNT solution into tube **1**. Then, transfer 10 µL of assay buffer into tubes **2** to **16**.
4. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **16** to get an equal volume of 10 µL for all samples.
5. Add 10 µL of 2 nM labeled A.1.1.1-Dy-654 to each tube from **16** to **1** and mix by pipetting.
6. Load capillaries directly.

## D1. MST System/Capillaries

Monolith Pico (NanoTemper Technologies GmbH)
Capillaries Monolith NT.115 (MO-K022, NanoTemper Technologies GmbH)

## D2. MST Software

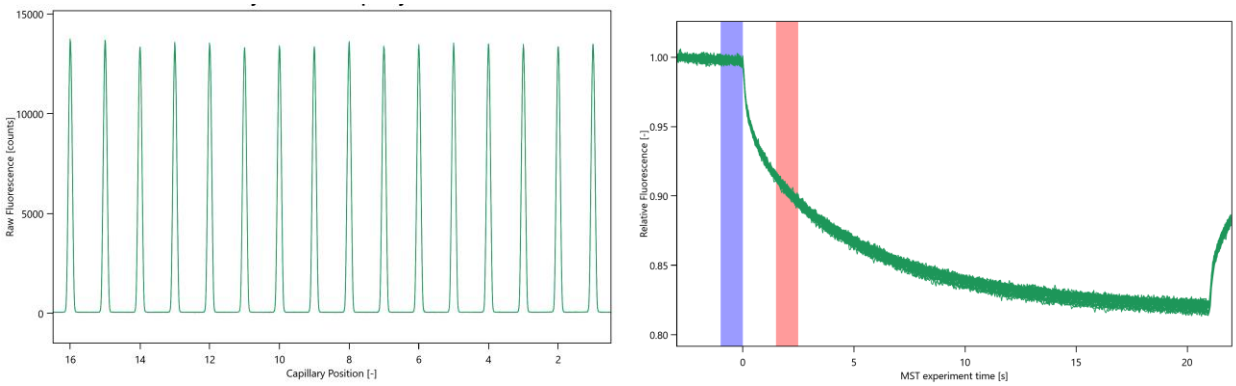
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

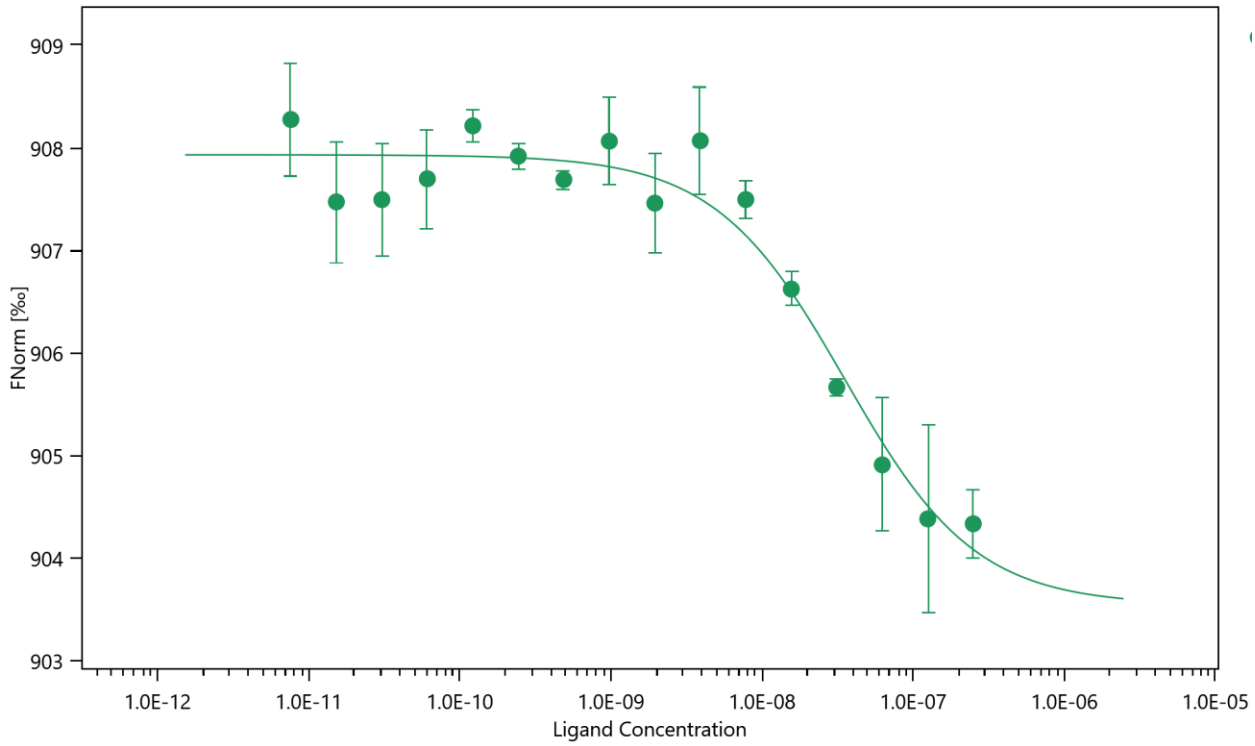
## D3. MST Experiment (Buffer/Concentrations/Temperature/MST Power/Excitation Power)

137 mM NaCl, 9.6 mM phosphate, 0.1 % BSA, pH 7.4
1 nM A.1.1.1-Dy654 | 7.6 pM – 500 nM TNT | 22°C | Medium MST Power | 30 % Excitation Power

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

$K_D$ = 37 ± 7 nM



## D5. Supporting Results

Paul et al., Biosensors. 10(8): 89 (2020)

## E. Contributors

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