Monolith Protocol MO-P-073

# Antibody – Morphine (competitive assay)

Heroin is a drug with one of the highest mortality rates. Therefore, there is an urgent need to develop alternative heroin abuse treatments. Recently, vaccines have been explored as a potential treatment modality for substances of abuse because they do not produce unwanted neurological side effects and have the potential to be utilized as preventive therapeutics against drug overdose. MicroScale Thermophoresis (MST) is a reliable method for measuring antibody binding affinities with a potential for use in vaccine development. Here, we show how the affinity of antibodies to heroin and its major metabolites such as morphine can be detected in a competitive MST assay using a fluorescent heroin analog. Moreover, the assay can even be used to determine affinities of polyclonal antibodies in sera.

antibody – small molecule interaction | heroin hapten | vaccines | competitive assay

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## A1. Target/Fluorescent Molecule

Anti-morphine antibody (ab1060)

## A2. Molecule Class/Organism

Monoclonal antibody
*Mouse (Mus musculus)*

## A3. Sequence/Formula

N/A

## A4. Purification Strategy/Source

Protein G purified
Abcam (Cambridge, MA, USA)
ab1060

## A5. Stock Concentration/Stock Buffer

1 mg/mL | ~6.7 µM
pH 7.4

## A6. Molecular Weight/Extinction Coefficient

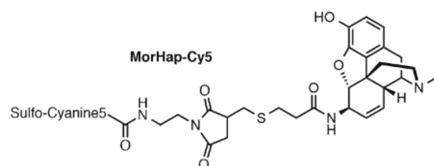
~150 kDa

## A7. Dilution Buffer

10 mM $Na_2HPO_4$, 1.8 mM $KH_2PO_4$, 2.7 mM KCl, 137 mM NaCl, pH 7.4, 0.05% Tween® 20
(Dulbecco’s phosphate buffered saline, DPBS-Tween)

## A8. Labeling Strategy

Competitive binding assay with MorHap-Cy5[1], a fluorescent heroin analog.




## A9. Labeling Procedure

1. Mix 100 µL of MorHap-Cy5 (1 nM) and 100 µL of ab1060 (40 nM) to obtain 200 µL of a 0.5 nM MorHap-Cy5, 20 nM ab1060 solution.[2]
2. Incubate for 20 minutes at room temperature in the dark.

## A10. Labeling Efficiency

N/A

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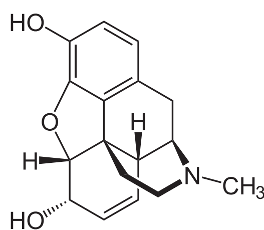
## B1. Ligand/Non-Fluorescent Binding Partner

Morphine




## B2. Molecule Class/Organism

Opiate

## B3. Sequence/Formula

$C_{17}H_{19}NO_3$

## B4. Purification Strategy/Source

Lipomed (Cambridge, MA, USA)

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[1] For the synthesis of MorHap-Cy5, see Torres et al., Analytical and Bioanalytical Chemistry (2018) 410:3885–3903.

[2] As the $K_d$ between MorHap-Cy5 and ab1060 is ~5 nM (c.f. section D4), a final concentration of 10 nM ab1060 is sufficient for complex formation, but still low enough for accurate $K_d$ determination of the morphine – ab1060 interaction.

## B5. Stock Concentration/Stock Buffer

1 mg/mL | 3.5 mM
Methanole

## B6. Molecular Weight/Extinction Coefficient

285.34 Da

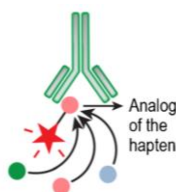
## B7. Serial Dilution Preparation

### Direct binding assay

1. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of a 3.2 µM solution of ab1060 into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
2. 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.
3. Add 10 µL of MorHap-Cy5 (0.5 nM) to each tube from **16** to **1** and mix by pipetting.
4. Incubate for 20 minutes at room temperature in the dark before loading capillaries.

### Competitive binding assay

1. Mix 2.3 µL of the 3.5 mM morphine stock solution with 998 µL of dilution buffer to obtain 1 mL of a 8 µM morphine solution.
2. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 8 µM morphine solution into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
3. 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.
4. Add 10 µL of the 0.5 nM MorHap-Cy5, 20 nM ab1060 solution to each tube from **16** to **1** and mix by pipetting.
5. Incubate for 20 minutes at room temperature in the dark before loading capillaries.



## D1. MST System/Capillaries

Monolith NT.115$^{Pico}$ Red (NanoTemper Technologies GmbH)
Capillaries Monolith NT.115 (MO-K022, NanoTemper Technologies GmbH)

## D2. MST Software

MO.Control v1.6 | MO.Affinity Analysis 2.2.4 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

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

### Direct binding assay

10 mM $Na_2HPO_4$, 1.8 mM $KH_2PO_4$, 2.7 mM KCl, 137 mM NaCl, pH 7.4, 0.05% Tween® 20
0.25 nM MorHap-Cy5 | 3.2 µM – 97 pM ab1060 | 25°C | low MST power | 20% excitation power

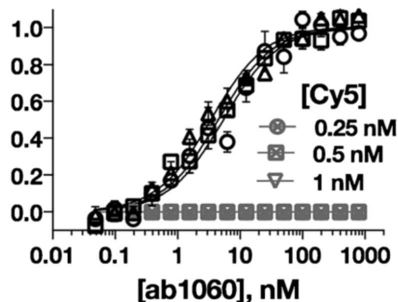
### Competitive binding assay

10 mM $Na_2HPO_4$, 1.8 mM $KH_2PO_4$, 2.7 mM KCl, 137 mM NaCl, pH 7.4, 0.05% Tween® 20
0.25 nM MorHap-Cy5, 10 nM ab1060 | 4 µM – 122 pM morphine | 25°C | low MST power | 20% excitation power

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

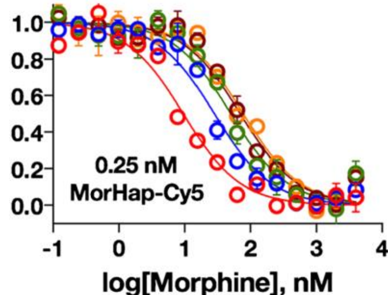
### Direct binding assay

$K_d$ = 5.29 ± 1.67 nM (MorHap-Cy5 – ab1060)




### Competitive binding assay

$EC_{50}$ = 28.2 ± 1.00 nM | $K_i$ = 6.87 ± 2.99[3] (ab1060 – morphine)




[3] For calculation of $K_i$, see also the NanoTemper 'FAQ Competitive Binding Assay'.

## D5. Reference Results/Supporting Results

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|---|---|
| $K_d = 1.97 \pm 0.17$ nM | Equilibrium dialysis with ultra-performance liquid chromatography/ tandem mass spectrometry (ED-UPLC/MS/MS)<br>Torres et al., Analytical and Bioanalytical Chemistry (2018) 410:3885–3903 |
| $K_d = 2$ nM | UV-VIS<br>Abcam |

## E. Contributors

Oscar B. Torres[4], Wyatt Strutz[5], Andreas Langer[6]

[4] Laboratory of Adjuvant and Antigen Research, Walter Reed Army Institute of Research, Silver Spring, MD, USA
[5] NanoTemper Technologies, Inc., Cambridge, MA, USA
[6] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com