Monolith Protocol MO-P-064

# CCL19 – CCR7 Chemokine Receptor Peptide

Chemokines are small molecular weight proteins which bind to and activate G-protein-coupled receptors (GPCRs) and play a crucial role in cancer cell metastasis. CCR7 is a chemokine receptor which when expressed on the surface of a cell recognizes chemokine CCL19. Interaction of chemokine CCL19 and chemokine receptor CCR7 peptides is characterized as a model system for development of peptide-based PPI inhibitors.

**protein – peptide interaction | chemokine | His-tag**

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## A1. Target/Fluorescent Molecule

CCL19
uniprot.org/uniprot/Q99731

## A2. Molecule Class/Organism

Chemokine
*Homo sapiens (Human)*

## A3. Sequence/Formula

MALLLALSLL VLWTSPAPTL SGTNDAEDCC LSVTQKPIPG YIVRNFHYLL IKDGCRVPAV VFTTLRGRQL CAPPDQPWVE RIIQRLQRTS AKMKRRSS

## A4. Purification Strategy/Source

Recombinant, N-terminal His-tag, produced in E. coli
Creative Biomart, Shirley, USA
CCL19-721H

## A5. Stock Concentration/Stock Buffer

20 µg lyophilized powder
Phosphate-buffered saline (PBS), pH 7.4, 0.05% TWEEN® 20

## A6. Molecular Weight/Extinction Coefficient

10.3 kDa
14,230 $M^{-1}cm^{-1}$ ($\varepsilon_{280}$)

## A7. Dilution Buffer

Phosphate-buffered saline (PBS), pH 7.4, 0.05% TWEEN® 20

## A8. Labeling Strategy

Monolith His-Tag Labeling Kit RED-tris-NTA (MO-L008, NanoTemper Technologies GmbH)
1* 125 pmol RED-tris-NTA Dye

## A9. Labeling Procedure

1. Suspend 20 µg CCL19 in 100 µL of PBS to obtain a 19.5 µM solution.
2. Suspend 125 pmol RED-tris-NTA Dye in 25 µL of dilution buffer to obtain a 5 µM dye solution.
3. Prepare a 100 nM dye solution by mixing 2 µL of dye (5 µM) and 98 µL of dilution buffer.
4. Prepare a 20 nM dye solution by mixing 20 µL of dye (100 nM) and 80 µL of dilution buffer.
5. Prepare a 200 nM CCL19 solution by mixing 1 µL of 19.5 µM CCL19 and 99 µL of dilution buffer.
6. Mix 100 µL of CCL19 (200 nM) with 100 µL of dye (20 nM).
7. Incubate for 30 minutes at room temperature in the dark.

## A10. Labeling Efficiency

N/A

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## B1. Ligand/Non-Fluorescent Binding Partner

CCR7 peptide[1]

## B2. Molecule Class/Organism

Truncated chemokine receptor peptide
*Homo sapiens (Human)*

## B3. Sequence/Formula

DDYIGDNTTV-$NH_2$

## B4. Purification Strategy/Source

In-house synthesis[2]

## B5. Stock Concentration/Stock Buffer

500 µM
Phosphate-buffered saline (PBS), pH 7.4, 0.05% TWEEN® 20

[1] Exemplified here at the truncated version 10.2.
[2] See Fuchs et al., Angew. Chem. Int. Ed. 58 (21), 7138–7142 (2019) for further information.

## B6. Molecular Weight/Extinction Coefficient

1111 Da
1,490 $M^{-1}cm^{-1}$ ($\varepsilon_{280}$)

## B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 500 µM CCR7 peptide solution 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 labeled CCL19 (100 nM) to each tube from **16** to **1** and mix by pipetting.
4. Incubate for 15 minutes at room temperature in the dark before loading capillaries.

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## D1. MST System/Capillaries

Monolith NT.115$^{Pico}$ Red (NanoTemper Technologies GmbH)
Premium Capillaries Monolith NT.115 (MO-K025, NanoTemper Technologies GmbH)

## D2. MST Software

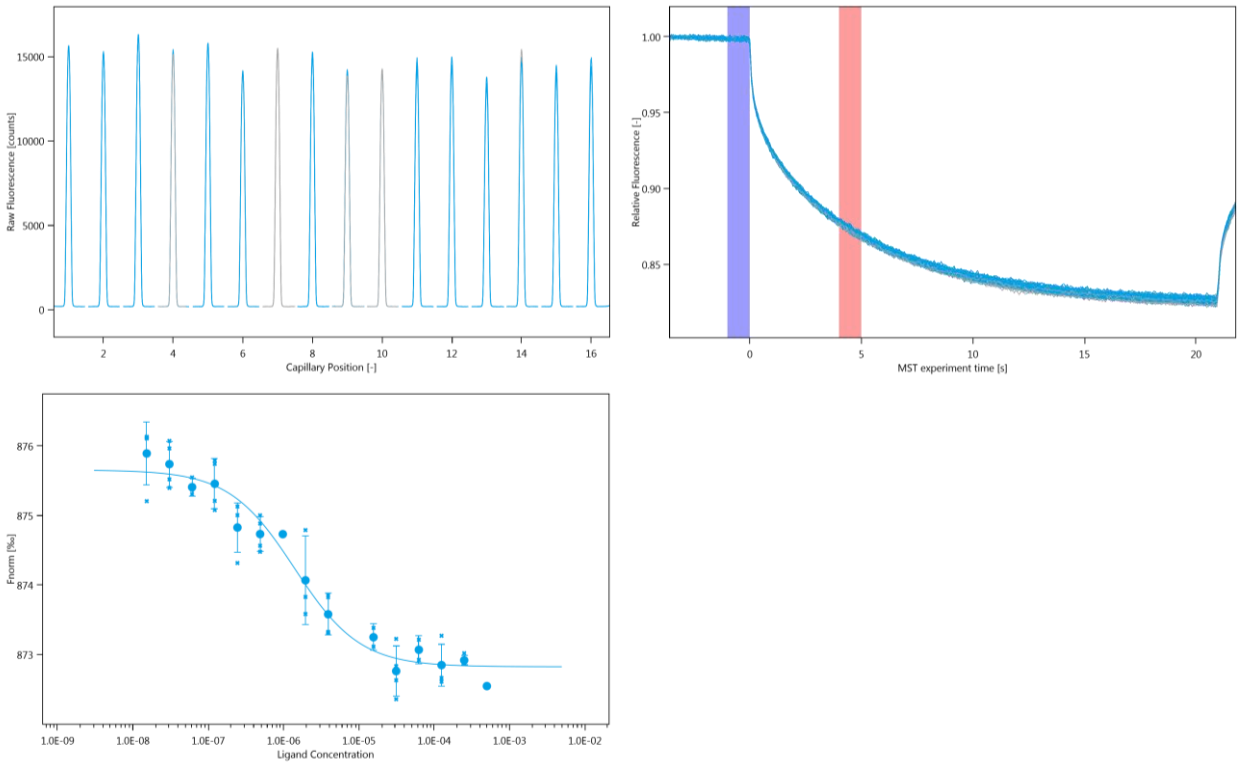
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software/

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

Phosphate-buffered saline (PBS), pH 7.4, 0.05% TWEEN® 20
50 nM CCL19 | 250 µM – 7.63 nM CCR7 | 25°C | medium MST power | 20% excitation power

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

$K_d$ = 1.4 µM Fuchs et al., Angew. Chem. Int. Ed. 58 (21), 7138–7142 (2019)[3]




## D5. Reference Results/Supporting Results

N/A

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## E. Contributors

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[3] In the publication, final concentrations of 10 nM CCL19 and 5 nM RED-tris-NTA Dye have been used.
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[5] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com