

## Monolith Protocol MO-P-015

# CREBBP – SGC-CBP30

CREB-binding protein, also known as CREBBP or CBP, is a transcriptional coactivator that carries out its function by activating transcription, where interaction with transcription factors is managed by one or more CREB domains. SGC-CBP30 is a selective inhibitor which binds to the bromodomain domain of CREBBP.

protein – small molecule interaction | bromodomain | inhibitor

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## A1. Target/Fluorescent Molecule

CREB-binding protein (CREBBP), bromodomain

[uniprot.org/uniprot/Q92793](https://uniprot.org/uniprot/Q92793)

## A2. Molecule Class/Organism

Transcriptional coactivator

*Homo sapiens (Human)*

## A3. Sequence/Formula

RKKIFKPEEL RQALMPTLEA LYRQDPESLP FRQPVDPQLL GIPDYFDIVK NPMDLSTIKR KLDTGQYQEP WQYVDDVWLM  
FNNAWLYNRK TSRVYKFCSK LAEVFEQEID PVMQSLG

## A4. Purification Strategy/Source

Crelux GmbH

## A5. Stock Concentration/Stock Buffer

10 mg/mL | 700  $\mu$ M

## A6. Molecular Weight/Extinction Coefficient

14 kDa

25,440  $\text{M}^{-1}\text{cm}^{-1}$  ( $\epsilon_{280}$ )

## A7. Dilution Buffer

50 mM HEPES, pH 7.6, 150 mM NaCl, 0.05% TWEEN® 20

## A8. Labeling Strategy

Monolith Protein Labeling Kit RED – NHS 2nd Generation (MO-L011, NanoTemper Technologies GmbH)

1\* Dye RED-NHS 2nd Generation (10  $\mu$ g) | 1\* B-Column

## A9. Labeling Procedure

1. Add 69  $\mu\text{L}$  of dilution buffer<sup>1</sup> to 1  $\mu\text{L}$  of 700  $\mu\text{M}$  CREBBP to obtain 70  $\mu\text{L}$  of a 10  $\mu\text{M}$  solution.
2. Add 12  $\mu\text{L}$  of DMSO to 10  $\mu\text{g}$  Dye RED-NHS 2nd Generation to obtain a  $\sim 1.25$  mM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
3. Mix 2.8  $\mu\text{L}$  of the 1.5 mM dye solution with 67.2  $\mu\text{L}$  of dilution buffer to obtain 70  $\mu\text{L}$  of a 50  $\mu\text{M}$  dye solution (5x protein concentration).
4. Mix CREBBP and dye in a 1:1 volume ratio (140  $\mu\text{L}$  final volume, 2% 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 140  $\mu\text{L}$  of the labeling reaction from step 4 to the center of the column and let sample enter the bed completely.
9. Add 600  $\mu\text{L}$  of dilution buffer after the sample has entered and discard the flow through.
10. Place column in a new collection tube, add 350  $\mu\text{L}$  of dilution buffer and collect the eluate.
11. Keep the labeled CREBBP ( $\sim 2$   $\mu\text{M}$ ) on ice in the dark.

## A10. Labeling Efficiency

Measurement of protein concentration and degree of labeling (DOL) using a NanoDrop™:

[nanotempertech.com/dol-calculator](https://nanotempertech.com/dol-calculator)

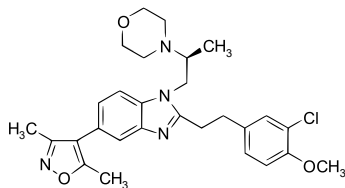
Absorbance $A_{280}$	0.061	Protein concentration	2.17 $\mu\text{M}$
Absorbance $A_{650}$	0.142	Degree-of-labeling (DOL)	0.33

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<sup>1</sup> CREBBP is not stable in the supplied Labeling Buffer NHS from the Protein Labeling kit MO-L011.

## B1. Ligand/Non-Fluorescent Binding Partner

SGC-CBP30



## B2. Molecule Class/Organism

Bromodomain inhibitor

## B3. Sequence/Formula

$C_{28}H_{33}ClN_4O_3$

## B4. Purification Strategy/Source

Sigma-Aldrich GmbH

[SML1133](#)

## B5. Stock Concentration/Stock Buffer

10.2 mg/mL | 20 mM

DMSO

## B6. Molecular Weight/Extinction Coefficient

509.04 Da

## B7. Serial Dilution Preparation

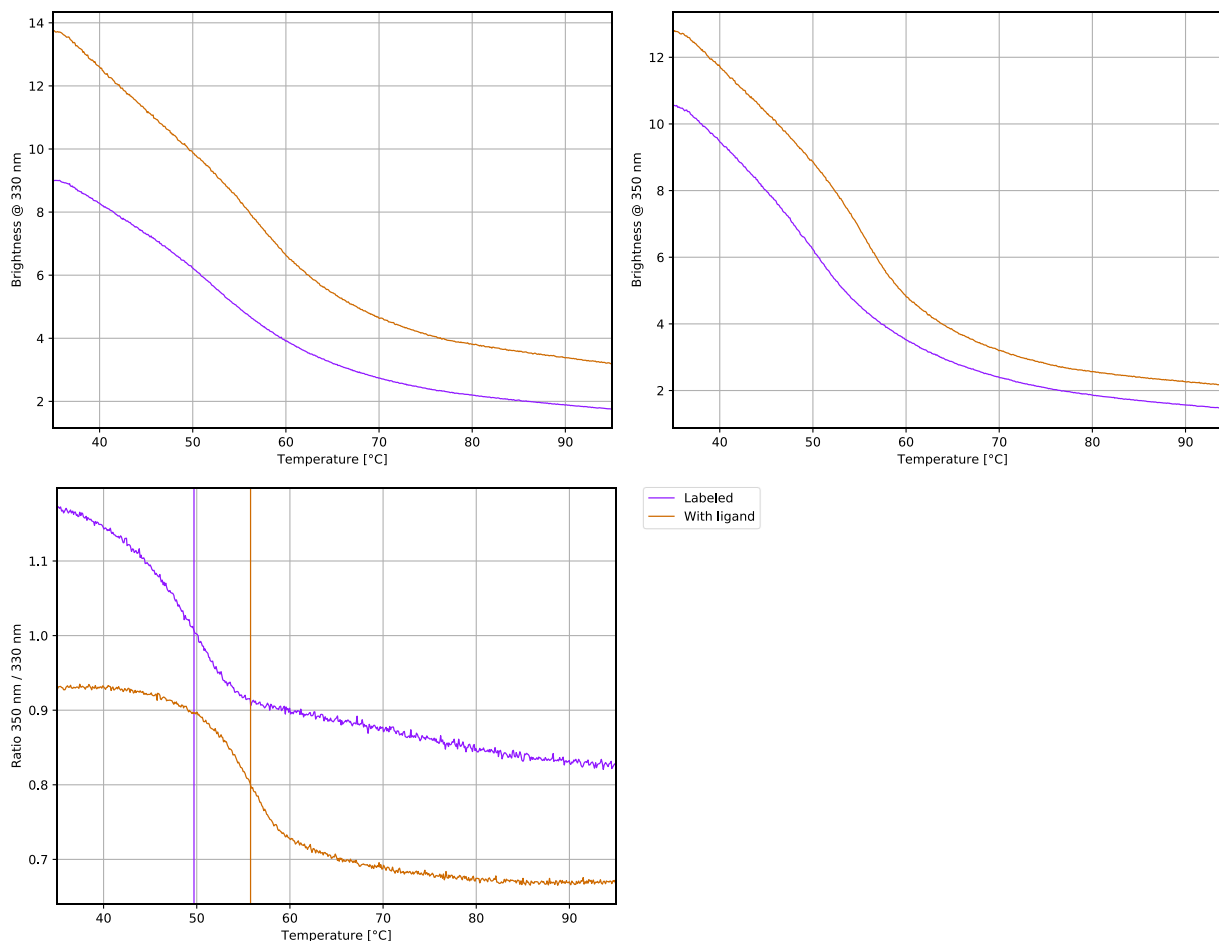
1. Mix 5  $\mu$ L of 20 mM SGC-CBP30 with 15  $\mu$ L of DMSO to obtain 20  $\mu$ L of a 5 mM solution.
2. Mix 2  $\mu$ L of the 5 mM SGC-CBP30 solution with 998  $\mu$ L of dilution buffer to obtain 1 mL of a 10  $\mu$ M SGC-CBP30 solution.
3. Mix 2  $\mu$ L of DMSO with 998  $\mu$ L of dilution buffer to obtain 1 mL of a 0.2% DMSO solution.
4. Prepare a PCR-rack with 16 PCR tubes. Transfer 20  $\mu$ L of the 10  $\mu$ M SGC-CBP30 solution into tube **1**. Then, transfer 10  $\mu$ L of the 0.2% DMSO solution into tubes **2** to **16**.
5. Prepare a 1:1 serial dilution by transferring 10  $\mu$ L from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10  $\mu$ L from tube **16** to get an equal volume of 10  $\mu$ L for all samples.
6. Mix 1  $\mu$ L of labeled CREBBP with 199  $\mu$ L of dilution buffer to obtain 200  $\mu$ L of ~10 nM CREBBP.
7. Add 10  $\mu$ L of CREBBP (~10 nM) to each tube from **16** to **1** and mix by pipetting.
8. Incubate for 20 minutes on ice before loading capillaries.

## C. Applied Quality Checks

Validation of structural integrity and functionality of labeled CREBBP using Tycho NT.6:

[nanotempertech.com/tycho](https://nanotempertech.com/tycho)

Labeled	5 $\mu$ L of B-Column eluate ( $\sim 2 \mu$ M) + 5 $\mu$ L of dilution buffer containing 0.2% DMSO	$T_i = 49.7^\circ\text{C}^2$
With ligand	5 $\mu$ L of B-Column eluate ( $\sim 2 \mu$ M) + 5 $\mu$ L of 10 $\mu$ M SGC-CBP30	$T_i = 55.8^\circ\text{C}$



## D1. MST System/Capillaries

Monolith NT.115<sup>Pico</sup> 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](https://nanotempertech.com/monolith-mo-control-software)

<sup>2</sup> Due to the low thermal stability, always keep on ice when not used.

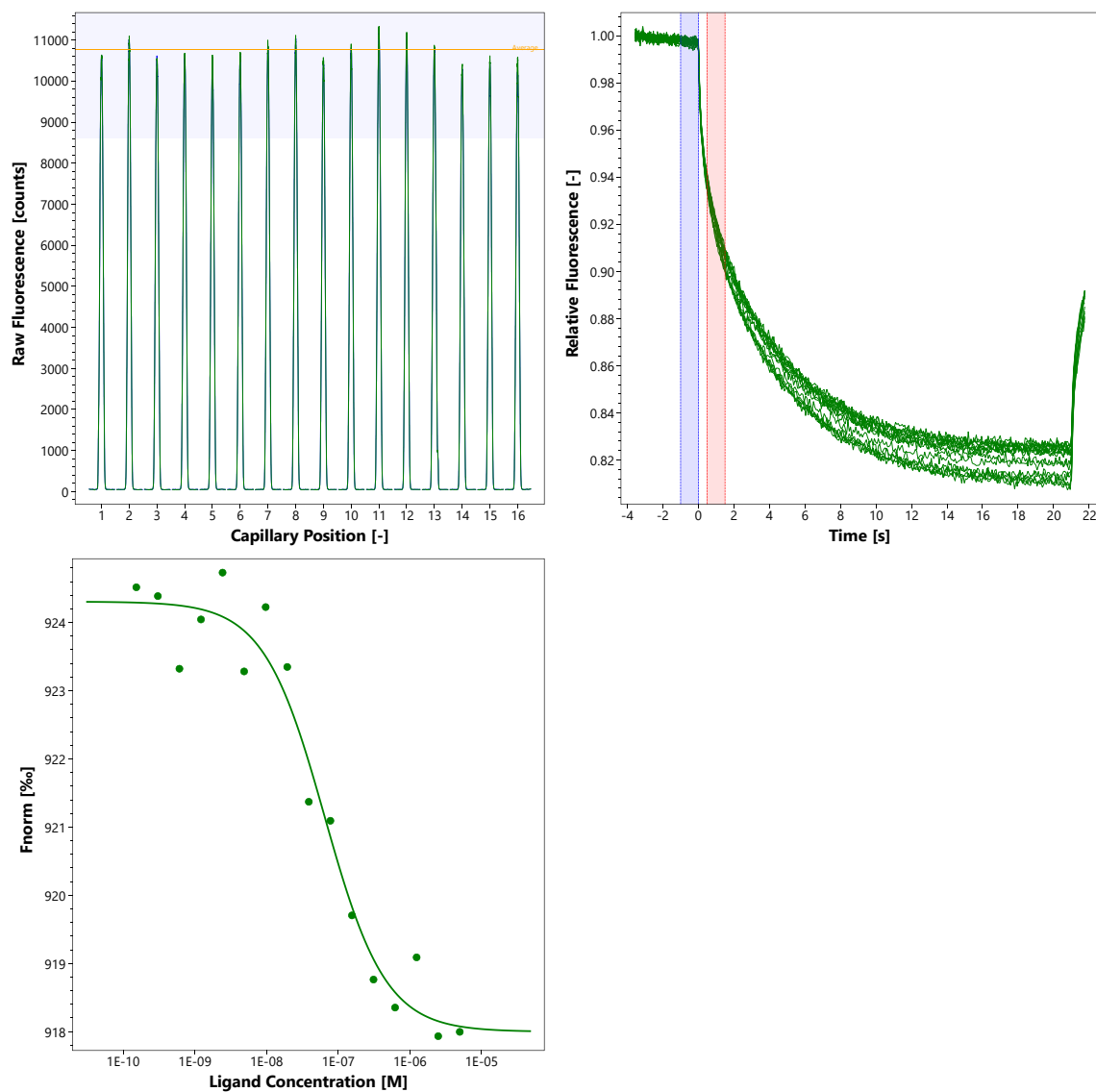
### D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

50 mM HEPES, pH 7.6, 150 mM NaCl, 0.1% DMSO<sup>3</sup>, 0.05% TWEEN® 20

5 nM CRIBBP | 5 µM – 153 pM SGC-CBP30 | 22°C | medium MST power | 20% excitation power

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

$K_d = 62.5$  nM



<sup>3</sup> Dimethyl sulfoxide (DMSO) itself is an inhibitor of bromodomains at the concentrations commonly used for compound solubilization as it mimics the acetylated lysine motif that is the canonical ligand recognized by bromodomains. Hence, DMSO concentrations > 0.1% can interfere with the assay ([Philpott, 2011](#)).

## D5. Reference Results/Supporting Results

$K_d = 26 \text{ nM}$	Isothermal Titration Calorimetry (ITC) <a href="#">Hammitzsch et al., PNAS 112(34), 10768–10773, 2015</a>
$K_d = 38 \text{ nM}$	BioLayer Interferometry (BLI) <a href="#">Hammitzsch et al., PNAS 112(34), 10768–10773, 2015</a>

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## E. Contributors

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