Monolith Protocol MO-P-014

# HSP90 – ADP

Heat shock protein 90 (Hsp90) is an ATP-dependent, ubiquitously expressed and highly abundant chaperone that aids in folding, stabilizing and degrading various other proteins, including kinases, steroid hormone receptors and transcription factors. By repeated ATP hydrolysis-coupled rounds of substrate binding and release, it helps both newly synthesized and misfolded proteins to find their native fold. Since a lot of Hsp90-dependent proteins are strongly upregulated in tumor cells, mammalian Hsp90 remains a promising target for anti-cancer drugs.

**protein – small molecule interaction | chaperone**

---

## A1. Target/Fluorescent Molecule

Heat shock protein HSP90-alpha (Hsp90)
uniprot.org/uniprot/P07900

## A2. Molecule Class/Organism

Heat shock protein 90 (Hsp90 family)
*Homo sapiens (Human)*

## A3. Sequence/Formula

```
GSMPEETQTQ DQPMEEEEVE TFAFQAEIAQ LMSLIINTFY SNKEIFLREL ISNSSDALDK IRYESLTDPS KLDSGKELHI
NLIPNKQDRT LTIVDTGIGM TKADLINNLG TIAKSGTKAF MEALQAGADI SMIGQFGVGF YSAYLVAEKV TVITKHNDDE
QYAWESSAGG SFTVRTDTGE PMGRGTKVIL HLKEDQTEYL EERRIKEIVK KHSQFIGYPI TLFVEKERDK EVSDDEAEEK
EDKEEEKEKE EKESEDKPEI EDVGSDEEEE KKDGDKKKKK KIKEKYIDQE ELNKTKPIWT RNPDDITNEE YGEFYKSLTN
DWEDHLAVKH FSVEGQLEFR ALLFVPRRAP FDLFENRKKK NNIKLYVRRV FIMDNCEELI PEYLNFIRGV VDSEDLPLNI
SREMLQQSKI LKVIRKNLVK KCLELFTELA EDKENYKKFY EQFSKNIKLG IHEDSQNRKK LSELLRYYTS ASGDEMVSLK
DYCTRMKENQ KHIYYITGET KDQVANSAFV ERLRKHGLEV IYMIEPIDEY CVQQLKEFEG KTLVSVTKEG LELPEDEEEK
KKQEEKKTKF ENLCKIMKDI LEKKVEKVVV SNRLVTSPCC IVTSTYGWTA NMERIMKAQA LRDNSTMGYM AAKKHLEINP
DHSIIETLRQ KAEADKNDKS VKDLVILLYE TALLSSGFSL EDPQTHANRI YRMIKLGLGI DEDDPTADDT SAAVTEEMPP
LEGDDDTSRM EEVD
```

## A4. Purification Strategy/Source

Expressed in *E. coli* RIL, $His_6$-tagged
Crelux GmbH

## A5. Stock Concentration/Stock Buffer

4.82 mg/mL | 56.8 µM
20 mM Tris-HCl, pH 8.0, 250 mM NaCl, 1 mM TCEP

## A6. Molecular Weight/Extinction Coefficient

84.8 kDa
59,250 $M^{-1}cm^{-1}$ ($\varepsilon_{280}$)

## A7. Dilution Buffer

50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 10 mM $MgCl_2$, 0.05% TWEEN® 20

## A8. Labeling Strategy

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

## A9. Labeling Procedure

1. Add 75 µL of Labeling Buffer NHS to 25 µL of 56.8 µM Hsp90 to obtain 100 µL of a 14.2 µM solution.
2. Use the A-Column to perform a buffer exchange into Labeling Buffer NHS.
   a. Invert A-Column to suspend slurry and twist off bottom (twist slightly in both directions).
   b. Loosen the cap of the column and place it in a 1.5 mL microcentrifuge collection tube.
   c. Centrifuge at **1500 × g** for **1 min** to remove excess liquid.
   d. Add 300 µL of Labeling Buffer NHS and centrifuge at **1500 × g** for **1 min** (3x).
   e. Place 100 µL of the 14.2 µM Hsp90 solution in the center of the resin.
   f. Place the sample in a **new** microcentrifuge collection tube and centrifuge at **1500 × g** for **2 min**.

   The collected flow-through should yield around 100 µL of ~10 µM Hsp90 (70 – 80% recovery).
3. 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.
4. Mix 5 µL of the 600 µM dye solution with 95 µL of Labeling Buffer NHS to obtain 100 µL of a 30 µM dye solution (3x protein concentration).
5. Mix Hsp90 and dye in a 1:1 volume ratio (200 µL final volume, 2.5% final DMSO concentration).
6. Incubate for 30 minutes at room temperature in the dark.
7. 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.
8. 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.
9. Add 200 µL of the labeling reaction from step 5 to the center of the column and let sample enter the bed completely.
10. Add 400 µL of dilution buffer after the sample has entered and discard the flow through.
11. Place column in a new collection tube, add 500 µL of dilution buffer and collect the eluate.
12. Keep the labeled Hsp90 (~2 µM) on ice in the dark.

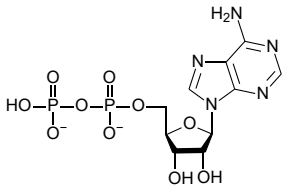
## A10. Labeling Efficiency

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

| | | | |
|---|---|---|---|
| Absorbance $A_{280}$ | 0.140 | Protein concentration | 2.03 µM |
| Absorbance $A_{650}$ | 0.490 | Degree-of-labeling (DOL) | 1.24 |

## B1. Ligand/Non-Fluorescent Binding Partner

Adenosine diphosphate (ADP)



## B2. Molecular Class/Organism

Nucleotide diphosphate

## B3. Sequence/Formula

$C_{10}H_{15}N_5O_{10}P_2$

## B4. Purification Strategy/Source

Sigma-Aldrich GmbH
A2754

## B5. Stock Concentration/Stock Buffer

42.7 mg/mL | 100 mM
$ddH_2O$

## B6. Molecular Weight/Extinction Coefficient

427.20 Da

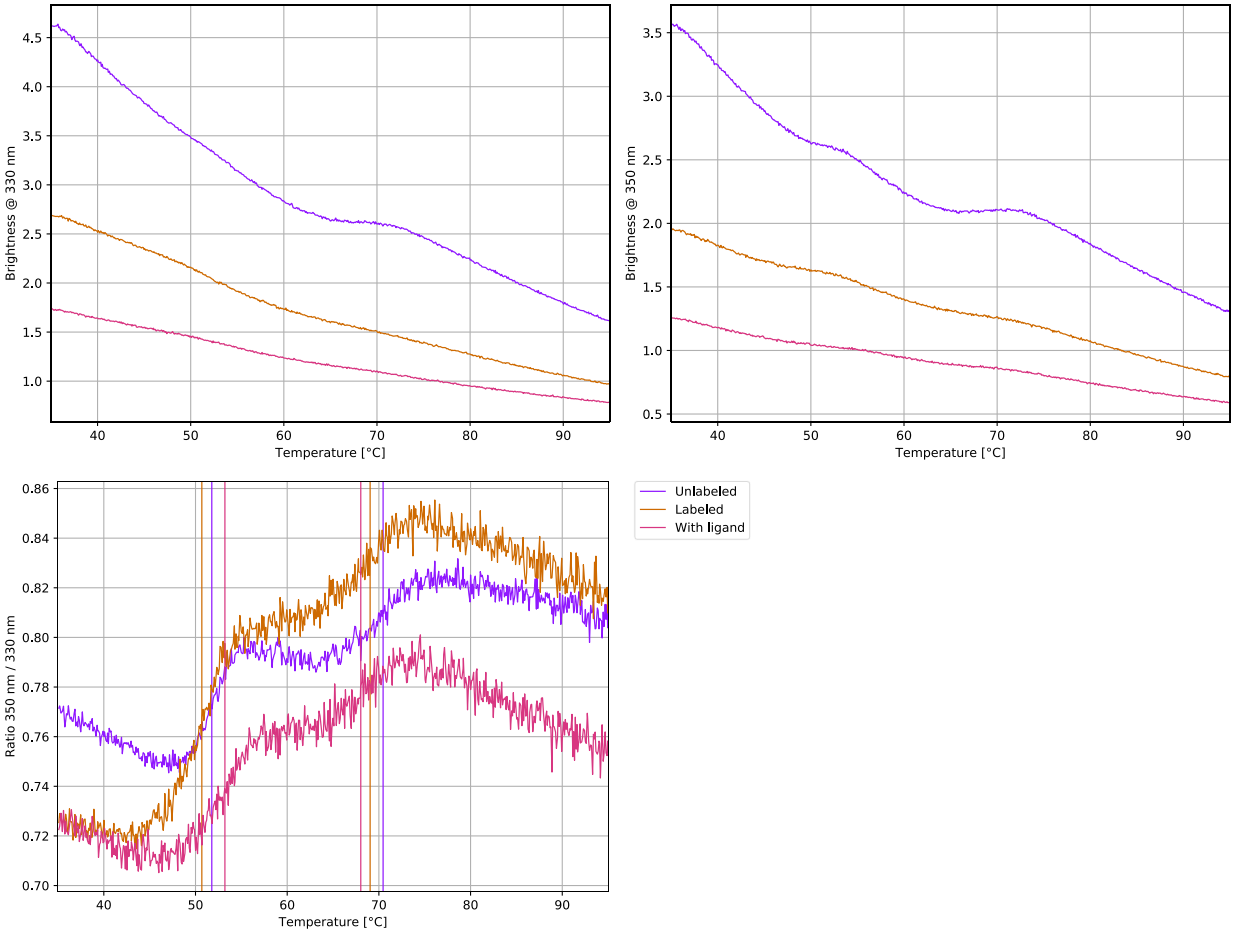
## B7. Serial Dilution Preparation

1. Add 27 µL of dilution buffer to 3 µL of the 100 mM ADP stock to obtain 30 µL of a 10 mM solution.
2. Add 180 µL of dilution buffer to 20 µL of $ddH_2O$ to obtain 200 µL of diluted dilution buffer.
3. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 10 mM ADP solution into tube **1**. Then, transfer 10 µL of diluted dilution 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. Mix 4 µL of labeled Hsp90 (~2 µM) with 196 µL of dilution buffer to obtain 200 µL of ~40 nM labeled Hsp90.
6. Add 10 µL of labeled Hsp90 (~40 nM) to each tube from **16** to **1** and mix by pipetting.
7. Incubate for 5 minutes at room temperature in the dark before loading capillaries.

## C. Applied Quality Checks

Validation of structural integrity and functionality of labeled Hsp90 using Tycho NT.6:
nanotempertech.com/tycho

| | | |
|---|---|---|
| Unlabeled | 10 µL of 2 µM Hsp90 | $T_{i,1}$ = 51.8°C \| $T_{i,2}$ = 70.5°C |
| Labeled | 5 µL of B-Column eluate (~2 µM) + 5 µL of dilution buffer | $T_{i,1}$ = 50.7°C \| $T_{i,2}$ = 69.0°C |
| With ligand | 5 µL of B-Column eluate (~2 µM) + 5 µL of 4 mM ADP | $T_{i,1}$ = 53.2°C \| $T_{i,2}$ = 68.0°C |




## D1. MST System/Capillaries

Monolith NT.115 Red (NanoTemper Technologies GmbH)
Premium Capillaries Monolith NT.115 (MO-K025, NanoTemper Technologies GmbH)

## D2. MST Software

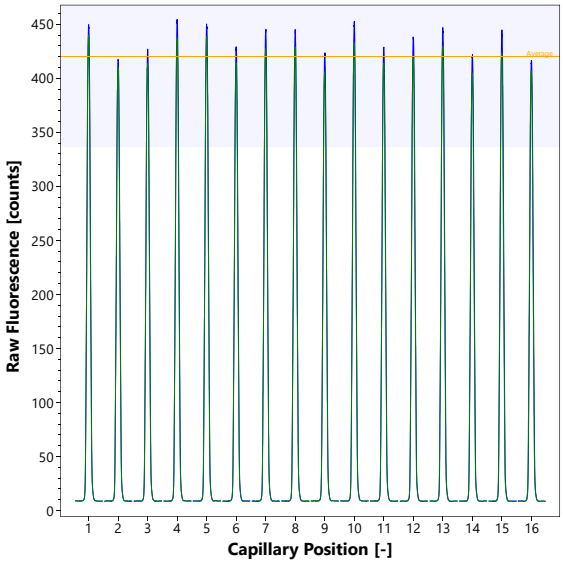
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

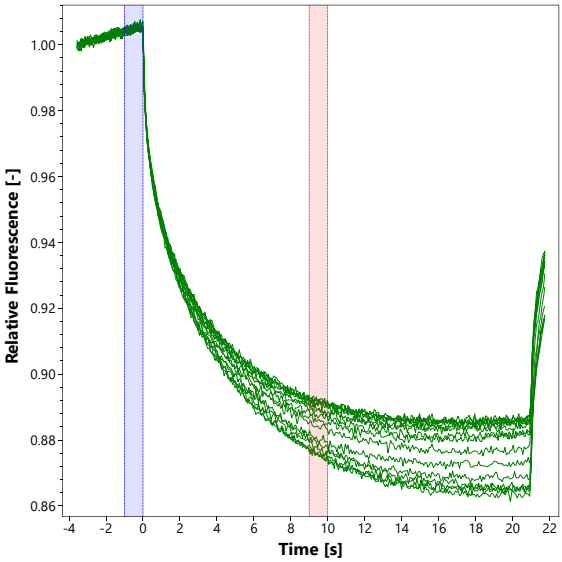
## D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

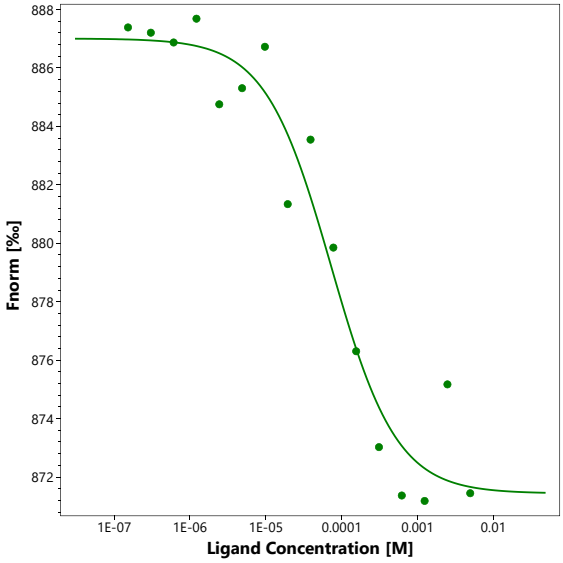
50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 10 mM $MgCl_2$, 0.05% TWEEN® 20
20 nM Hsp90 | 5 mM ADP – 153 nM | 25°C | medium MST power | 80% excitation power

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

$K_d$ = 72.9 µM




## D5. Reference Results/Supporting Results

$K_d$ = 29 µM Isothermal Titration Calorimetry (ITC)
Prodromou et al., Cell 90(1), 65-75 (1997)

## E. Contributors

Timm Hassemer[1]

[1] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com