Monolith X Protocol MOX-P-109

Herceptin – HER2

HER2 is a member of the human epidermal growth factor receptor (HER/EGFR/ERBB) family. Amplification or over-expression of this oncogene has been shown to play an important role in the development and progression of certain aggressive types of breast cancer. In recent years, the protein has become an important biomarker and target of therapy for many breast cancer patients. Herceptin (Trastuzumab) is a monoclonal antibody used to treat breast cancer.

protein – protein | antibody

A1. Target/Fluorescent Molecule

Herceptin (Trastuzumab)

A2. Molecule Class/Organism

Monoclonal antibody, IgG1

A3. Sequence/Formula

Heavy chain

```
EVQLVESGGG LVQPGGSLRL SCAASGFNIK DTYIHWVRQA PGKGLEWVAR IYPTNGYTRY ADSVKGRFTI SADTSKNTAY
LQMNSLRAED TAVYYCSRWG GDGFYAMDYW GQGTLVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS
WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG
PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK
EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG
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Light chain

```
DIQMTQSPSS LSASVGDRVT ITCRASQDVN TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS RFSGSRSGTD FTLTISSLQP
EDFATYYCQQ HYTTPPTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ
ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
```

A4. Purification Strategy/Source

N/A

A5. Stock Concentration/Stock Buffer

120 mg/mL | 825 µM

A6. Molecular Weight/Extinction Coefficient

145.5 kDa
225,000 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

20 mM HEPES, pH 7.4, 150 mM NaCl, 0.01% Pluronic® F-127

A8. Labeling Strategy

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

A9. Labeling Procedure

1. Prepare 50 µL of a 20 µM Herceptin solution.
2. Add 25 µL of DMSO to 10 µg RED-NHS 2nd Generation dye to obtain a ~600 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
3. Mix 5 µL of the 600 µM dye solution with 45 µL Labeling Buffer NHS to obtain 50 µL of a 60 µM dye solution (3x protein concentration).
4. Mix Herceptin and dye in a 1:1 volume ratio (100 µL final volume, ~5% 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 100 µL of the labeling reaction from step 5 to the center of the column and let sample enter the resin bed completely.
9. Add 500 µL of dilution buffer after the sample has entered and discard the flow through.
10. Place column in a new collection tube, add 400 µL of dilution buffer and collect the eluate.
11. Centrifuge the eluate at 15,000 rpm and 4°C for 15 minutes to remove aggregates. Then, carefully transfer the supernatant into a fresh tube and mix well by pipetting.
12. Prepare 10 µL aliquots of the labeled Herceptin (~2.5 µM) and store at -80°C.

A10. Labeling Efficiency

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

Absorbance A_{280}	0.607	Protein concentration	~2.59 µM
Absorbance A_{650}	0.627	Degree-of-labeling (DOL)	~1.24

B1. Ligand/Non-Fluorescent Binding Partner

ErbB-2 (HER2)
uniprot.org/uniprot/P04626

B2. Molecule Class/Organism

Receptor tyrosine-protein kinase
Homo sapiens (Human)

B3. Sequence/Formula

```
TQVCTGTDMK LRLPASPETH LDMLRHLYQG CQVVQGNLEL TYLPTNASLS FLQDIQEVQG YVLIAHNQVR QVPLQRLRIV
RGTQLFEDNY ALAVLDNGDP LNNTTPVTGA SPGGLRELQL RSLTEILKGG VLIQRNPQLC YQDTILWKDI FHKNNQLALT
LIDTNRSRAC HPCSPMCKGS RCWGESSEDC QSLTRTVCAG GCARCKGPLP TDCCHEQCAA GCTGPKHSDC LACLHFNHSG
ICELHCPALV TYNTDTFESM PNPEGRYTFG ASCVTACPYN YLSTDVGSCT LVCPLHNQEV TAEDGTQRCE KCSKPCARVC
YGLGMEHLRE VRAVTSANIQ EFAGCKKIFG SLAFLPESFD GDPASNTAPL QPEQLQVFET LEEITGYLYI SAWPDSLPDL
SVFQNLQVIR GRILHNGAYS LTLQGLGISW LGLRSLRELG SGLALIHHNT HLCFVHTVPW DQLFRNPHQA LLHTANRPED
ECVGEGLACH QLCARGHCWG PGPTQCVNCS QFLRGQECVE ECRVLQGLPR EYVNARHCLP CHPECQPQNG SVTCFGPEAD
QCVACAHYKD PPFCVARCPS GVKPDLSYMP IWKFPDEEGA CQPCPINCTH SCVDLDDKGC PAEQRASPLT
```

B4. Purification Strategy/Source

Recombinant, His-tagged (C-terminus)
Sino Biological
10004-H08H

B5. Stock Concentration/Stock Buffer

0.71 mg/mL | 10 µM
PBS

B6. Molecular Weight/Extinction Coefficient

71 kDa
65,000 $M^{-1}cm^{-1}$ (ε_{280})

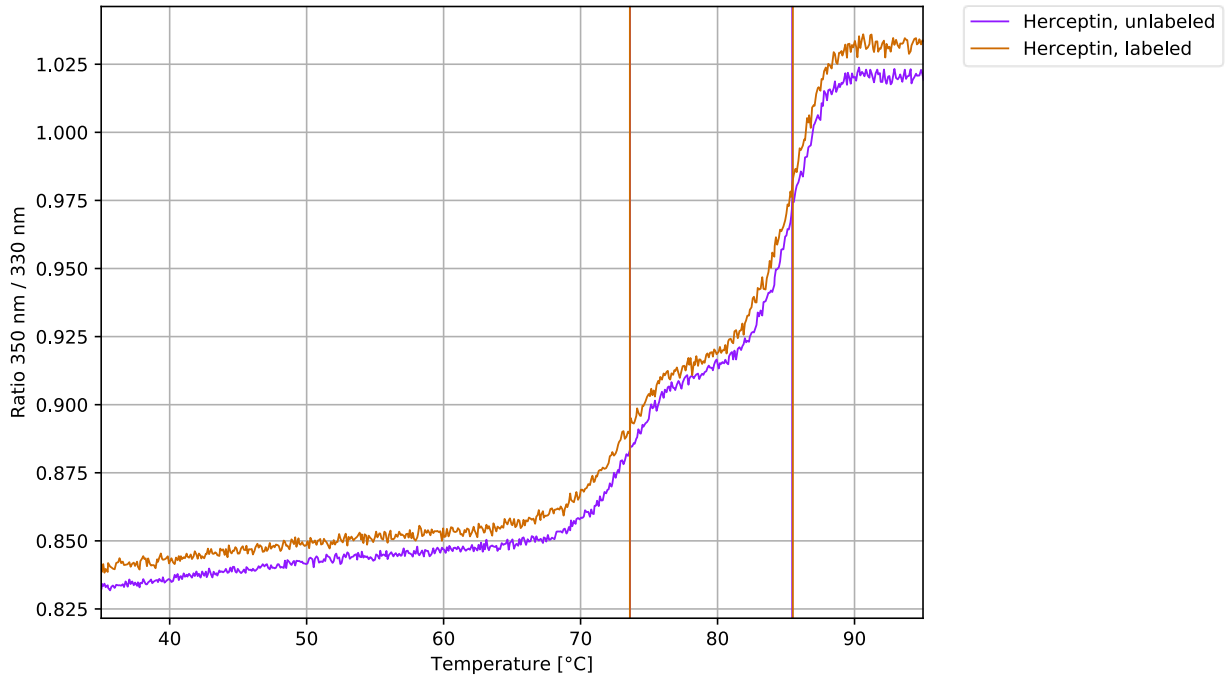
B7. Serial Dilution Preparation

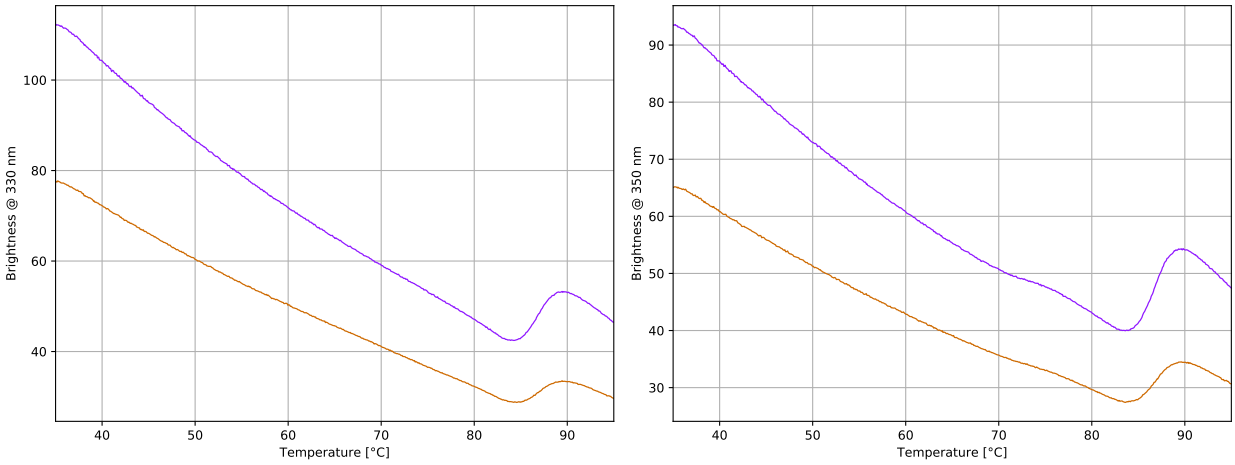
1. Prepare a PCR-rack with 16 PCR tubes. Mix 2 µL of 10 µM HER2 with 18 µL of dilution buffer in tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
2. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **16** to get an equal volume of 10 µL for all samples.
3. Mix 2 µL of the labeled Herceptin (~2.5 µM) with 998 µL of dilution buffer to obtain 1 mL of a ~5 nM Herceptin solution.
4. Add 10 µL of labeled Herceptin (~5 nM) to each tube from **16** to **1** and mix by pipetting.
5. Incubate for at least 2 hours at room temperature in the dark before loading capillaries.

C. Tycho

Validation of structural integrity of labeled Herceptin using Tycho NT.6:
nanotempertech.com/tycho

Herceptin, unlabeled	1.25 µL of 20 µM Herceptin + 8.75 µL of dilution buffer	$T_{i,1}$ = 73.6°C | $T_{i,2}$ = 85.4°C
Herceptin, labeled	10 µL of B-Column eluate (~2.5 µM)	$T_{i,1}$ = 73.6°C | $T_{i,2}$ = 85.5°C

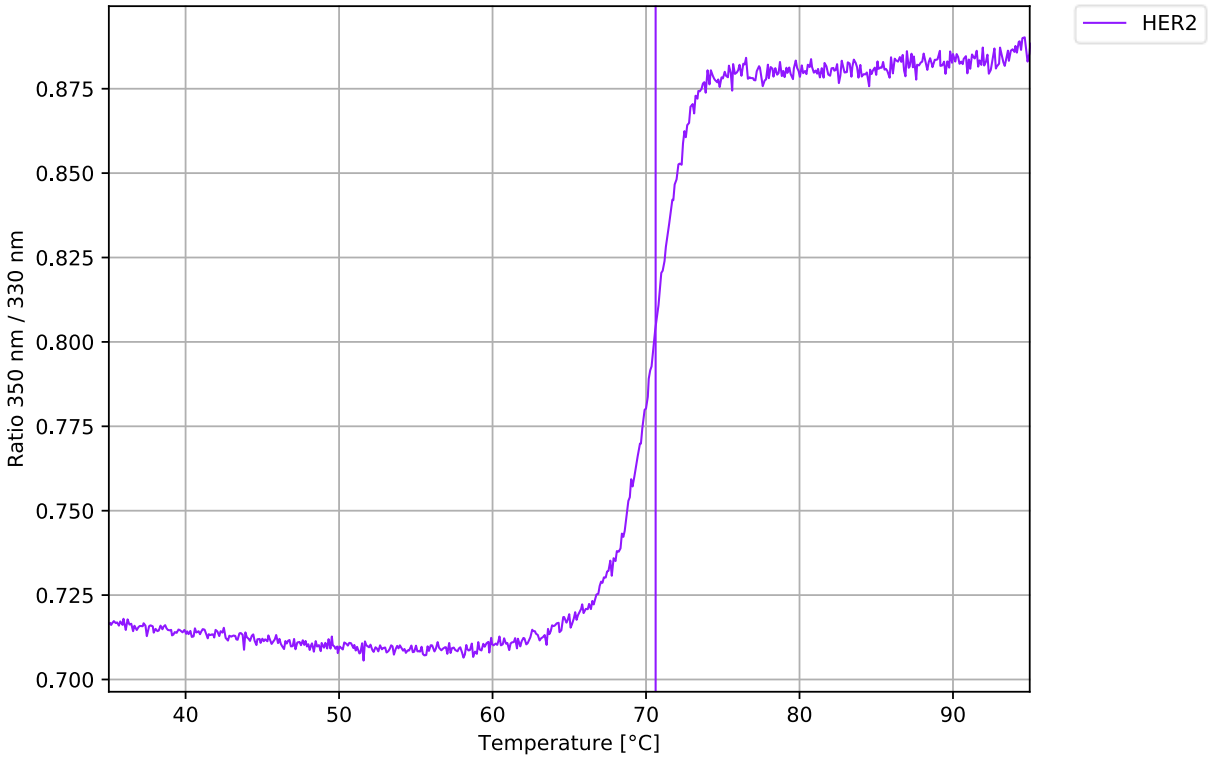


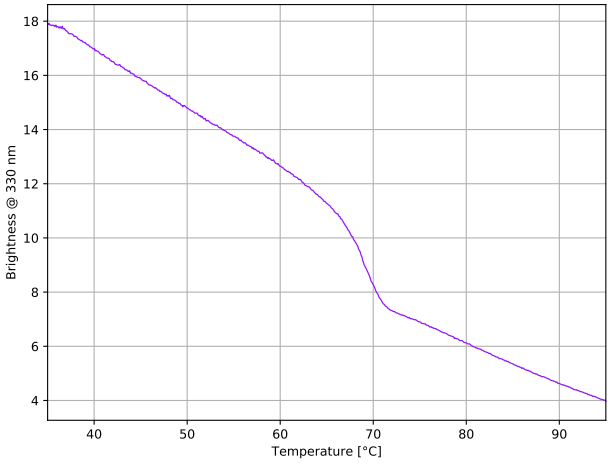


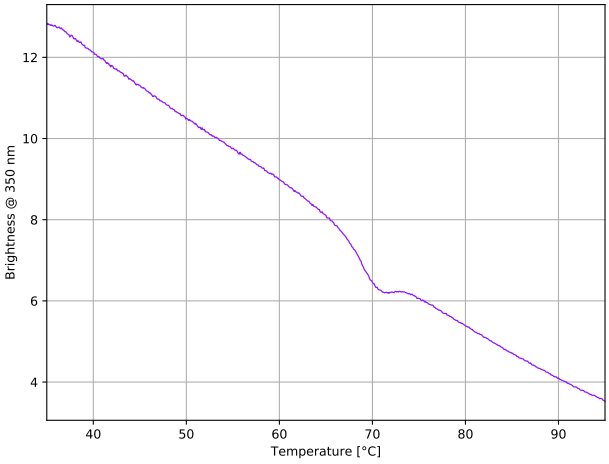
Validation of structural integrity of HER2 using Tycho NT.6:

nanotempertech.com/tycho

HER2	1 µL of 10 µM HER2 + 9 µL of dilution buffer	T_i = 70.6°C







D1. Monolith System/Capillaries

Monolith X (NanoTemper Technologies GmbH)
Monolith Premium Capillaries (MO-K025, NanoTemper Technologies GmbH)

D2. Monolith Software

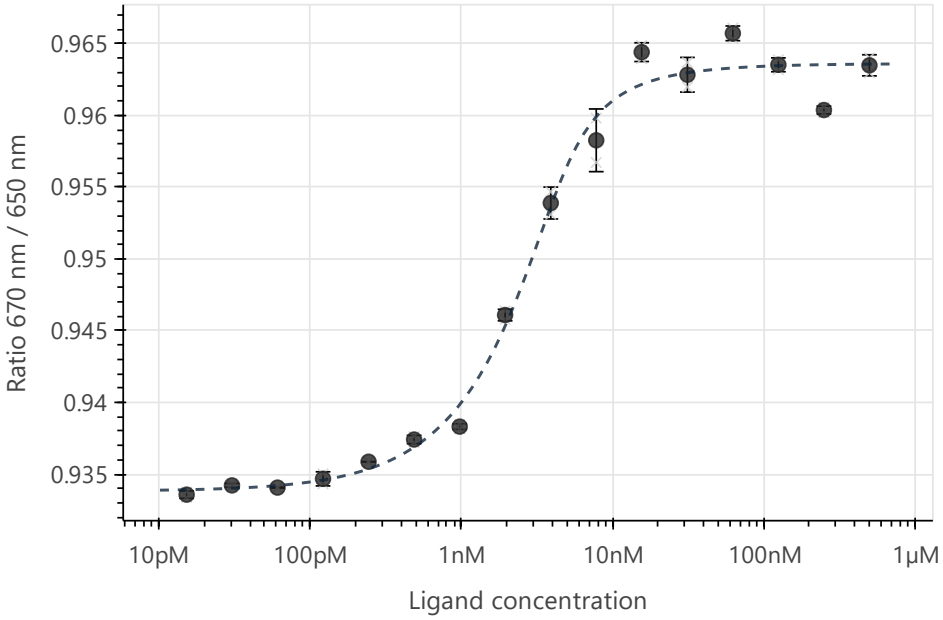
MO.Control v2.4.2 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

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

20 mM HEPES, pH 7.4, 150 mM NaCl, 0.01% Pluronic® F-127
2.5 nM Herceptin | 500 nM – 15.3 pM HER2 | 20°C | 100% excitation power

D4. Monolith Results (Dose Response)

K_d = 598 ± 202 pM (S/N = 22.0)



D5. Reference Results/Supporting Results

K_d = 1.3 nM (k_a = 1.2 × 10^5 $M^{-1}s^{-1}$, k_d = 1.6 × 10^{-4} s^{-1})	Surface Plasmon Resonance (SPR) Epa et al., PLOS One, 8(3), 2013
K_d = 0.4 nM (k_a = 2.8 × 10^5 $M^{-1}s^{-1}$, k_d = 1.2 × 10^{-4} s^{-1})	Bio-Layer Interferometry (BLI) Pedersen et al., Mol Cancer Ther, 14(3) 2015

E. Contributors

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