Monolith X Protocol MOX-P-117

# HER2 – Herceptin ADCs

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 this 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. Trastuzumab antibody-drug conjugates (ADCs) are a type of targeted cancer therapy that combines the specificity of monoclonal antibodies with the cytotoxic effects of chemotherapy drugs.

**protein – protein | antibody | ADCs**

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## A1. Target/Fluorescent Molecule

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

## A2. Molecule Class/Organism

Receptor tyrosine-protein kinase
*Homo sapiens (Human)*

## A3. 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
```

## A4. Purification Strategy/Source

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

## A5. Stock Concentration/Stock Buffer

1 mg
Lyophilized (PBS, pH 7.4)

## A6. Molecular Weight/Extinction Coefficient

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

## A7. Dilution Buffer

Phosphate-buffered saline (PBS), pH 7.4, 0.005% 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 µg) | 1* B-Column

## A9. Labeling Procedure

1. Resuspend 1 mg HER2 in 500 µL dd$H_2O$ to obtain a 2 mg/mL (28.2 µM) solution.
2. Prepare 10 µL aliquots and store at -80°C.
3. Mix 7.1 µL of 28.2 µM HER2 with 12.9 µL of dilution buffer to obtain 20 µL of 10 µM HER2.
4. Add 12.5 µL of DMSO to 10 µg RED-NHS 2nd Generation dye to obtain a ~1.2 mM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
5. Mix 0.5 µL of the 1.2 mM dye solution with 19.5 µL of dilution buffer to obtain 20 µL of a 60 µM dye solution (3x protein concentration).
6. Mix HER2 and dye in a 1:1 volume ratio (40 µL final volume, ~1.25% final DMSO concentration).
7. Incubate for 30 minutes at room temperature in the dark.
8. 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.
9. 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.
10. Add 40 µL of the labeling reaction from step 6 to the center of the column and let sample enter the resin bed completely.
11. Add 560 µL of dilution buffer after the sample has entered and discard the flow through.
12. Place column in a new collection tube, add 500 µL of dilution buffer and collect the eluate.
13. Prepare 20 µL aliquots of the labeled HER2 (~0.4 µM) and store at -80°C.

## A10. Labeling Efficiency

N/A

## B1. Ligand/Non-Fluorescent Binding Partner

Herceptin (Trastuzumab)

| | | |
|---|---|---|
| Trastuzumab emtansine (Kadcyla) | Trastuzumab deruxtecan (Enhertu) | Disitamab vedotin (RC48) |

## B2. Molecule Class/Organism

Monoclonal antibody, IgG1

## B3. 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
```

Light chain

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

## B4. Purification Strategy/Source

MedChemExpress

| | | |
|---|---|---|
| HY-P9921 | HY-138298A | HY-P9985 |

## B5. Stock Concentration/Stock Buffer

| | | |
|---|---|---|
| 5 mg/mL \| 34.3 µM | 5 mg/mL \| 34.3 µM | 5 mg/mL \| 34.3 µM |

## B6. Molecular Weight/Extinction Coefficient

145.5 kDa

225,000 $M^{-1}cm^{-1}$ ($\varepsilon_{280}$)

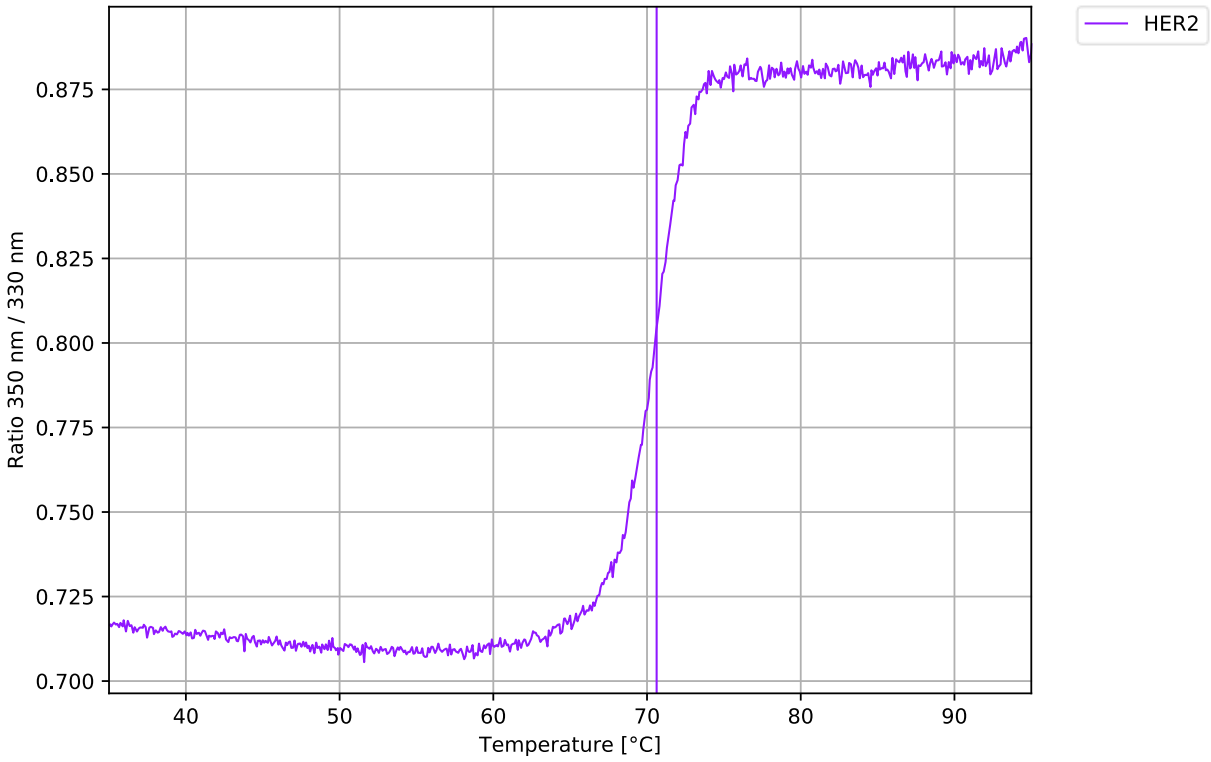
## B7. Serial Dilution Preparation

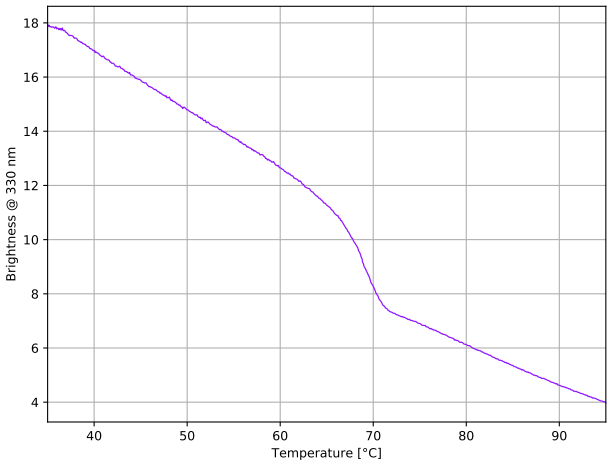
1. Mix 1 µL of 34.3 µM ADC with 170.5 µL of dilution buffer to obtain a 200 nM ADC solution.
2. Mix 8 µL of 400 nM labeled HER2 with 392 µL of dilution buffer to obtain 400 µL of an 8 nM HER2 solution.
3. Take a fresh PCR tube and mix 80 µL of dilution buffer with 80 µL of 8 nM HER2 to obtain 160 µL of 4 nM HER2.
4. Prepare a PCR-rack with 16 PCR tubes. Transfer 10 µL of 4 nM HER2 into tubes **2** to **16**. Then, mix 10 µL of 8 nM HER2 with 10 µL of 200 nM ADC in tube **1**.
5. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down.
6. Incubate for at least 30 minutes at room temperature in the dark before loading capillaries.

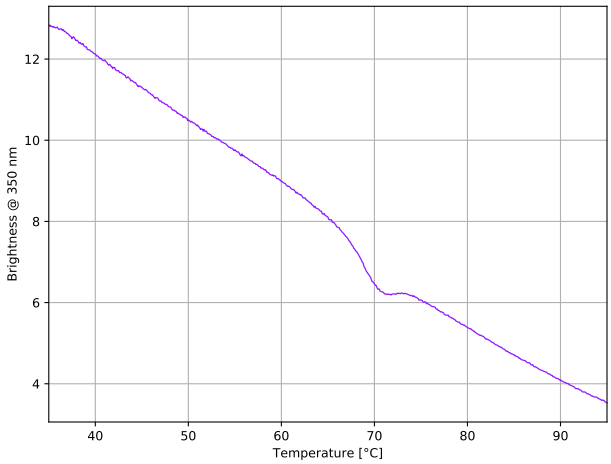
## C. Tycho

Validation of structural integrity of HER2 using Tycho NT.6:
nanotempertech.com/tycho

| | | |
|---|---|---|
| HER2 | 10 µL of 1 µM HER2 | $T_i$ = 70.6°C |



## D1. Monolith System/Capillaries

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

## D2. Monolith Software

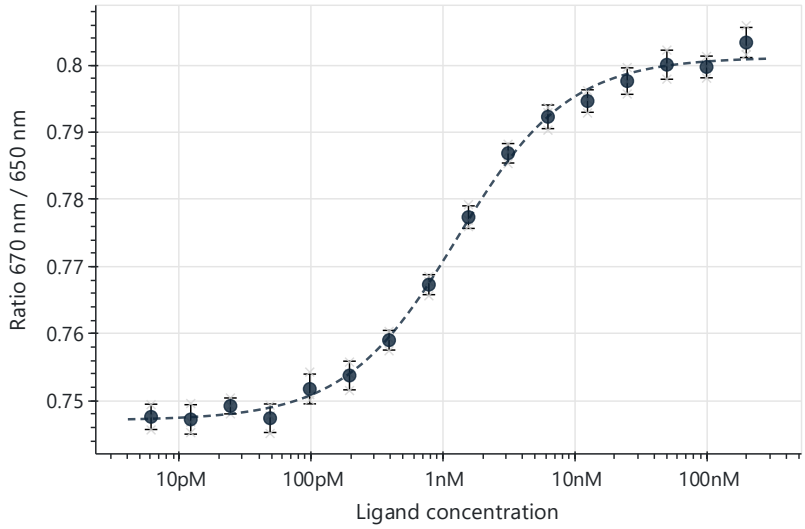
MO.Control v2.6.3 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

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

Phosphate-buffered saline (PBS), pH 7.4, 0.005% TWEEN® 20
4 nM HER2 | 100 nM[1] – 3.0 pM Trastuzumab ADC | 25°C | 100% excitation power

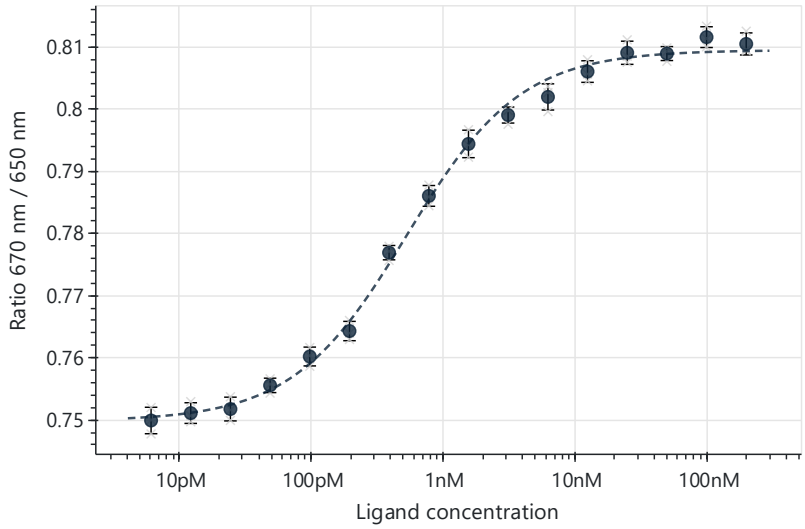
## D4. Monolith Results (Dose Response)

Trastuzumab | $K_d$ = 1.42 ± 0.1 nM (S/N = 49.1)

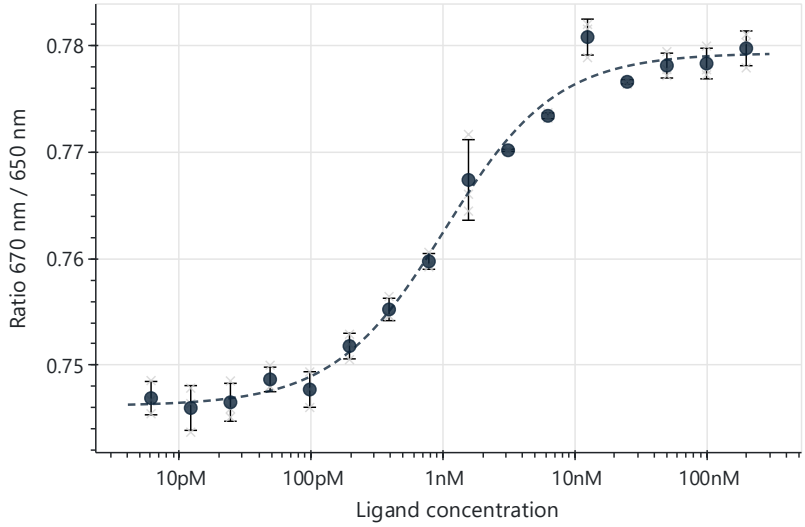



[1] 100 nM Trastuzumab corresponds to a ligand concentration of 200 nM (two antigen binding sites per antibody)

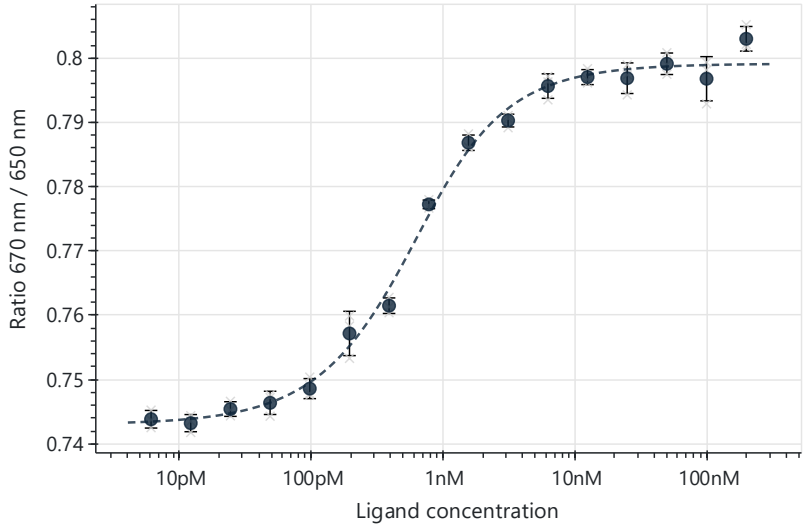
Trastuzumab emtansine | $K_d$ = 646 ± 49 pM (S/N = 42.6)




Disitamab vedotin | $K_d$ = 1.12 ± 0.15 nM (S/N = 24.6)




Trastuzumab deruxtecan | $K_d$ = 441 ± 55 pM (S/N = 33.0)



## D5. Reference Results/Supporting Results

| | |
|---|---|
| $K_d$ = 1.3 nM<br>($k_a = 1.2 \times 10^5\ M^{-1}s^{-1}$, $k_d = 1.6 \times 10^{-4}\ s^{-1}$) | Surface Plasmon Resonance (SPR)<br>Epo et al., PLOS One, 8(3), 2013 |
| $K_d$ = 0.4 nM<br>($k_a = 2.8 \times 10^5\ M^{-1}s^{-1}$, $k_d = 1.2 \times 10^{-4}\ s^{-1}$) | Surface Plasmon Resonance (SPR)<br>Pedersen et al., Mol Cancer Ther, 14(3) 2015 |

## E. Contributors

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