Monolith Protocol MO-P-043

Guanine Quadruplex DNA – 1H6 Monoclonal Antibody

Guanine quadruplex (G4) structures are higher order structures readily formed both *in vitro* and *in vivo* from single stranded guanine-rich RNA or DNA molecules. These structures are often found in the telomeric regions of chromosomes and play a regulatory role in various cellular pathways. 1H6 is a mouse monoclonal antibody which strongly binds to synthetic G4 DNA structures and can serve as a tool for detection of G4 structures *in situ.*

G-quadruplex DNA | antibody | stoichiometry

A1. Target/Fluorescent Molecule

G4 oligonucleotides

A2. Molecule Class/Organism

Synthetic oligonucleotides

A3. Sequence/Formula

$(T_4G_4)_2$	5' Cy5 TTT TGG GGT TTT GGG G 3'
$(T_4G_3)_2$	5' Cy5 TTT TGG GTT TTG GG 3'
$(T_3G_3)_2$	5' Cy5 TTT GGG TTT GGG 3'
$(T_3G_4)_2$	5' Cy5 TTT GGG GTT TGG GG 3'
MYC	5' Cy5 TGA GGG TGG GTA GGG TGG GTA A 3'
$(A_4G_4)_2$	5' Cy5 AAA AGG GGA AAA GGG G 3'
$(C_4G_4)_2$	5' Cy5 CCC CGG GGC CCC GGG G 3'
poly(T)	5' Cy5 TTT TTT TTT TTT TTT 3'

A4. Purification Strategy/Source

Purified with high-performance liquid chromatography (HPLC)
IDT(Leuven, Belgium)

Oligonucleotides were diluted to 10 µM in TE buffer (10 mM Tris-HCl, pH 7.5, 100 mM KCl, 1 mM EDTA). Following denaturation for 10 min at 95°C, G4 structures could form overnight by slowly cooling to room temperature in the heating block.

A5. Stock Concentration/Stock Buffer

100 µM
ddH_2O

A6. Molecular Weight/Extinction Coefficient

5'Cy5	Single stranded, linear $(T_4G_4)_2$
532.6 Da	5,005.26 Da
250,000 $M^{-1}cm^{-1}$ (ε_{648})	147,000 $M^{-1}cm^{-1}$ (ε_{260})

Single stranded sequences are used to prepare quadruplexes. Quadruplexes could be tetramolecular, bimolecular and unimolecular.

A7. Dilution Buffer

400 mM Na PBS, 0.5% BSA, 0.05% TWEEN® 20

A8. Labeling Strategy

5' Cy5 labeled

A9. Labeling Procedure

N/A

A10. Labeling Efficiency

HPLC-purified, 100% labeled DNA

B1. Ligand/Non-Fluorescent Binding Partner

1H6 monoclonal mouse antibody (mAb)

B2. Molecule Class/Organism

Monoclonal antibody

B3. Sequence/Formula

Heavy Chain	CDRH-1								CDRH-2								CDRH-3							
	27			30	35			38	56			59			62	65	105							
1H6	G	F	T	F	R	N	Y	W	I	R	L	K	S	D	N	Y	A	T	T	N	W	Y	Y	F	D	Y

Light Chain	CDRL-1										CDRL-2				CDRL-3									
	27					32	34			38	56			65	105									
1H6	Q	S	L	L	Y	S	N	G	K	T	Y	L	V	S	V	Q	G	T	H	F	P	L	T	

Adapted from Henderson et al.[1]

[1] Henderson et al., Nucleic Acids Research, 42 (2), 2014, 860–869

B4. Purification Strategy/Source

Intermolecular Guanine Quadruplex DNA Mouse, Monoclonal Antibody (1H6)
MédiMabs (Montreal, Canada)
MM-0265-P

B5. Stock Concentration/Stock Buffer

1 mg/mL | 6.45 µM
PBS

B6. Molecular Weight/Extinction Coefficient

150 kDa

B7. Serial Dilution Preparation

1. Mix 31 µL of the 6.45 µM 1H6 solution with 69 µL of dilution buffer to obtain 100 µL of a 2 µM solution.
2. Prepare a PCR-rack with 16 PCR tubes. Transfer 40 µL of the 2 µM 1H6 solution into tube **1**. Then, transfer 20 µL of dilution buffer into tubes **2** to **16**.
3. Prepare a 1:1 serial dilution by transferring 20 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 20 µL from tube **16** to get an equal volume of 20 µL for all samples.
4. Mix 2 µL of 10 µM 5'Cy5 G-quadruplex oligonucleotide with 398 µL of dilution buffer to obtain 400 µL of a 50 nM solution.
5. Add 20 µL of 50 nM 5'Cy5 G-quadruplex oligonucleotide to each tube from **16** to **1** and mix by pipetting.
6. Incubate tubes for 5 minutes at room temperature in the dark before loading capillaries.

Dilution series over wide range to narrow down point of saturation

1. Mix 8 µL of 10 µM 5'Cy5 G-quadruplex oligonucleotide with 8 µL of 10 µM unlabeled G-quadruplex oligonucleotide and 384 µL of dilution buffer to obtain 400 µL of a 400 nM solution.
2. Prepare a PCR-rack with 16 new PCR tubes. Transfer 40 µL of the 2 µM 1H6 solution into tube **1**. Then, transfer 20 µL of dilution buffer into tubes **2** to **16**.
3. Prepare a 1:1 serial dilution by transferring 20 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 20 µL from tube **16** to get an equal volume of 20 µL for all samples.
4. Add 20 µL of 400 nM 5'Cy5 G-quadruplex oligonucleotide to each tube from **16** to **1** and mix by pipetting.
5. Incubate tubes for 5 minutes at room temperature in the dark before loading capillaries.

Dilution series over small concentration range to precisely determine stoichiometry

1. Mix 15 µL of the 2 µM 1H6 solution with 123 µL of dilution buffer to obtain 138 µL of a 700 nM solution.
2. Prepare a PCR-rack with 16 PCR tubes. Transfer 16 µL, 15 µL, …, 2 µL, 1 µL of the 700 nM 1H6 solution into tubes **1** to **16**. Then, transfer 1 µL, 2 µL, …, 14 µL, 15 µL of dilution buffer into tubes **2** to **16** to get an equal volume of 16 µL for all samples.
3. Prepare a 1:1 serial dilution by transferring 16 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 16 µL from tube **16** to get an equal volume of 16 µL for all samples.
4. Add 16 µL of 400 nM 5'Cy5 G-quadruplex oligonucleotide to each tube from **16** to **1** and mix by pipetting.
5. Incubate tubes for 5 minutes at room temperature in the dark before loading capillaries.

D1. MST System/Capillaries

Monolith NT.115 Red (NanoTemper Technologies GmbH)
Capillaries Monolith NT.115 (MO-K022, NanoTemper Technologies GmbH)

D2. MST Software

MO.AffinityAnalysis v2.3 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith/#monolith-software

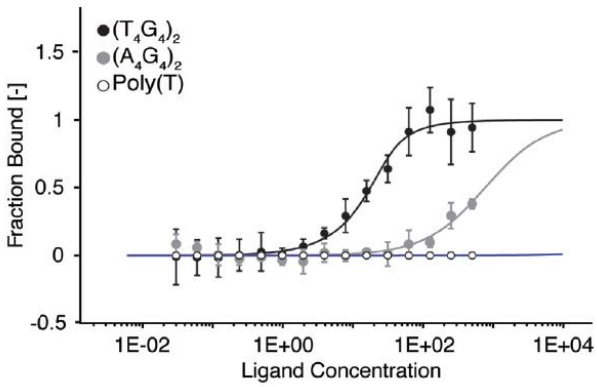
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

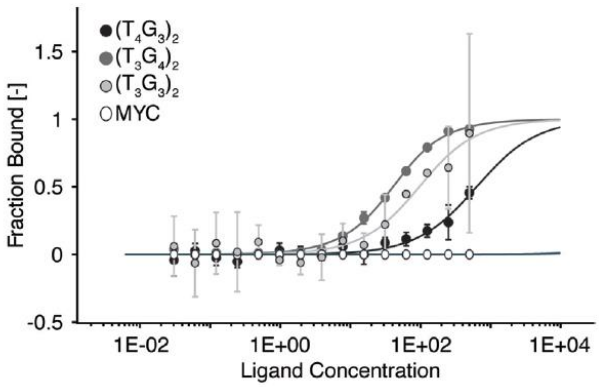
400 mM Na PBS, 0.5% BSA, 0.05% TWEEN® 20
25 nM G4s | 1 µM – 0.03 nM 1H6 antibody | 24°C | medium MST power | 80% excitation power
400 mM Na PBS, 0.5% BSA, 0.05% TWEEN® 20
200 nM G4s | 1 µM – 0.03 nM and 350 nM – 50 nM 1H6 antibody | 24°C | medium MST power | 40% excitation power

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

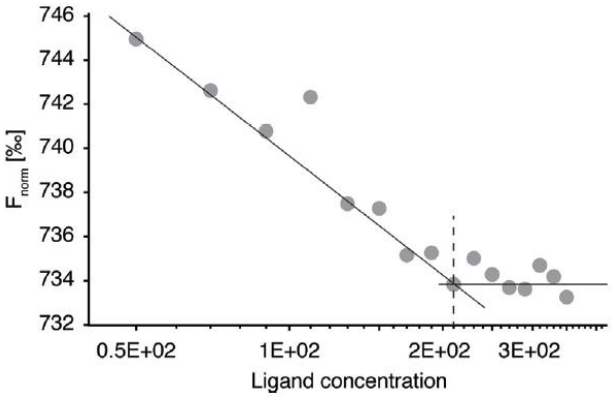
$(T_4G_4)_2$	K_d = 5 nM
$(T_4G_3)_2$	K_d = 620 nM
$(T_3G_3)_2$	K_d = 83 nM
$(T_3G_4)_2$	K_d = 28 nM
MYC	No binding
$(A_4G_4)_2$	K_d = 754 nM
$(C_4G_4)_2$	K_d = 184 nM
poly(T)	No binding

Kazemier et al., Nucleic Acids Research, 45 (10), 2017, 5913–5919





Intersection of linear fits[2] at ~215 nM of 1H6 (200 nM of G4S) → 1 : 1 stoichiometry



D5. Reference Results/Supporting Results

G-quadruplex DNA

Sen and Gilbert, Methods in Enzymology 211, 1992, 191-199 | Henderson et al., Nucleic Acids Research, 42 (2), 2014, 860–869

Hoffmann et al., Nucleic Acids Research, 44 (1), 2016, 152–163 | Kazemier et al., Nucleic Acids Research, 45 (10), 2017, 5913–5919

E. Contributors

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[2] Linear regression lines of the saturated and non-saturated data points were set manually.

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