We tug you to success

Analytical Strategies for dsRNA Detection in IVT mRNA

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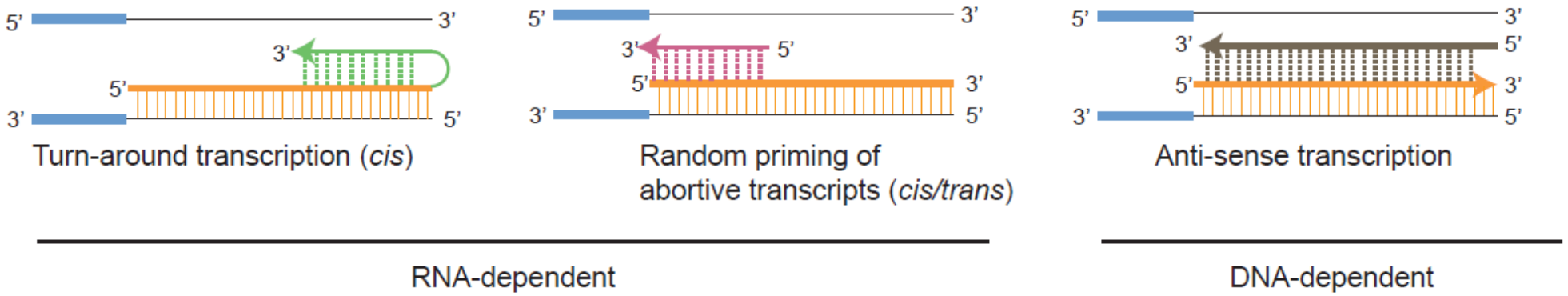
Possible Mechanisms of dsRNA Formation

- Self-priming via intramolecular base pairing
- Abortive transcript priming
- Template switching
- Promoter-independent transcription



DOI:10.1089/gen.39.12.15.

Analytical Methods for Detection of dsRNA

Immunological Assays

- Dot blot assay
- ELISA

Electrophoretic Methods

- Gel electrophoresis (native and denaturing)
- Capillary electrophoresis (microfluidic)

Chromatographic Techniques

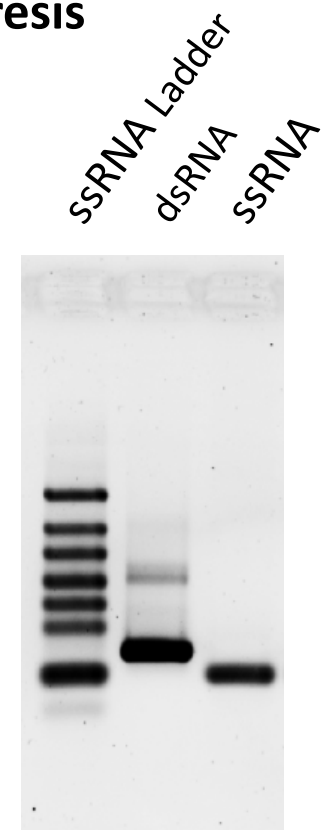
- RP-HPLC

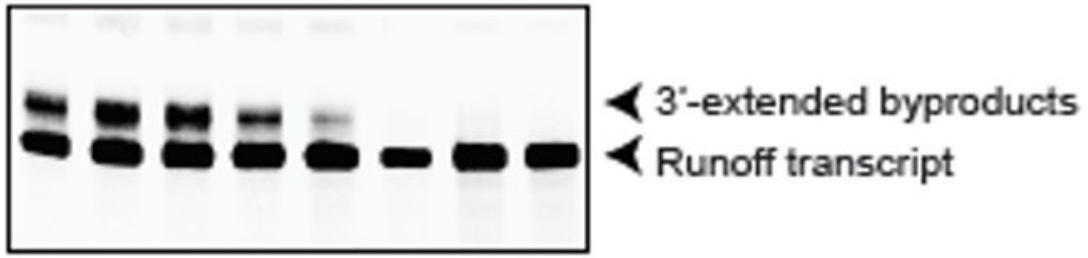
Mass Spectrometry

- LCMS

Electrophoretic Methods

Gel Electrophoresis





- **Can resolve dsRNA from ssRNA**
- **Low sensitivity**

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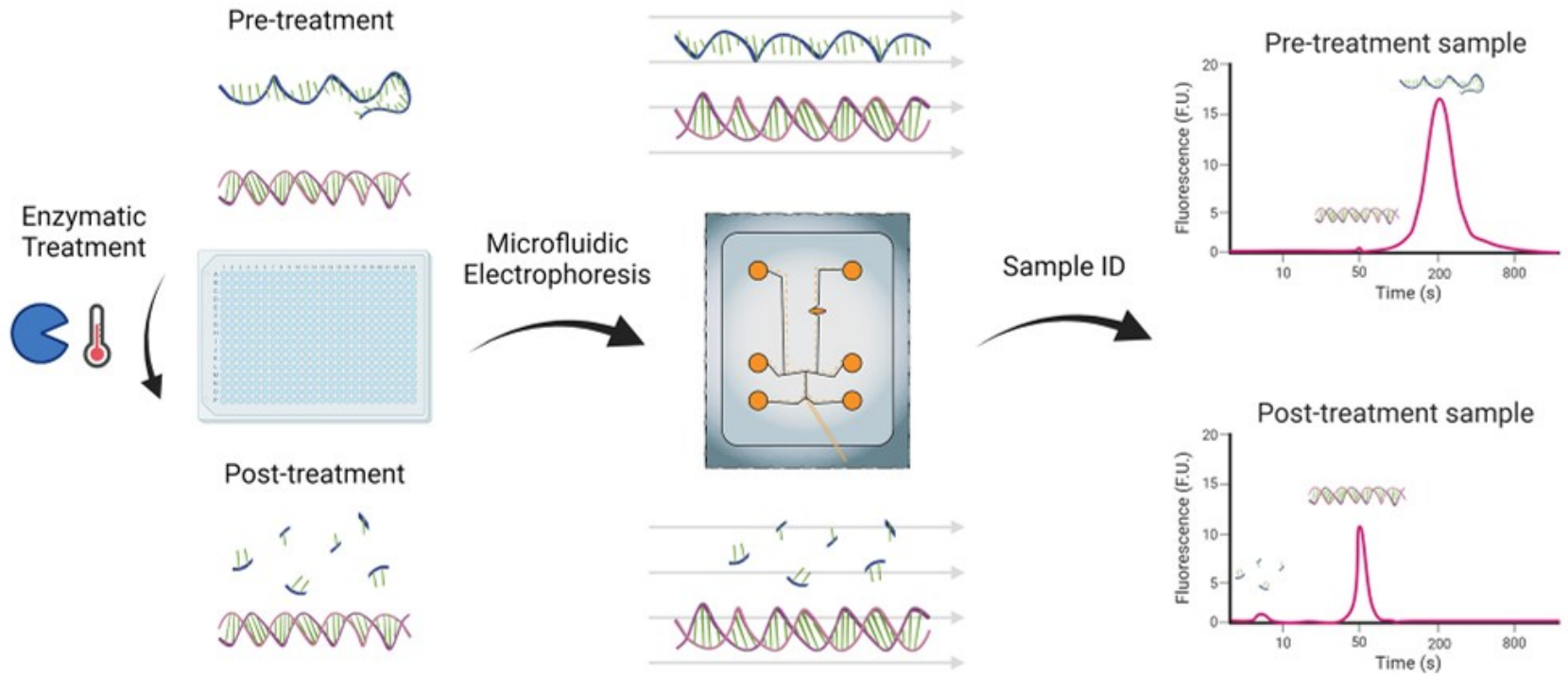
Electrophoretic Methods

Microfluidic Electrophoresis

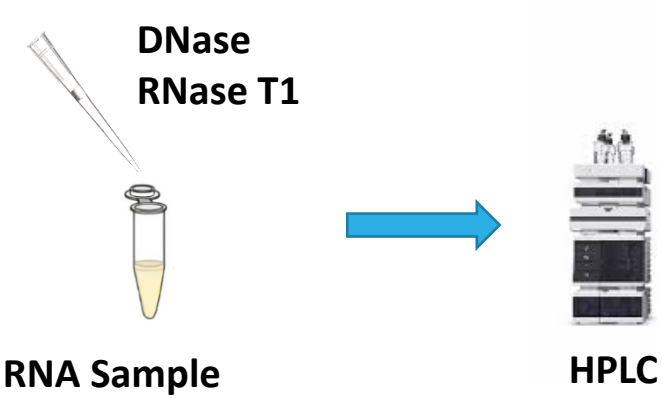


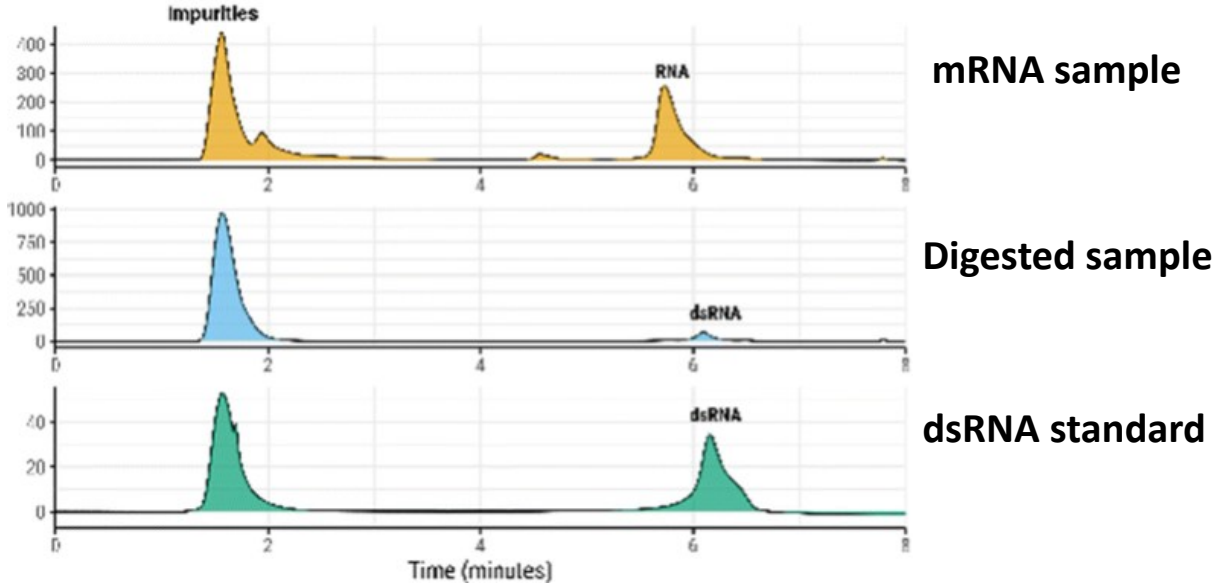
- **High-throughput**
- **Enzyme treatment**
- **LOD could be less than 1 μg/mL**

DOI:10.1039/d3an02157b

Chromatographic Techniques

RP-HPLC





- Enzyme treatment
- Separate according to hydrophobicity
- LOD could reach around 10 µg/mL

DOI: 10.1039/d4ay00560k

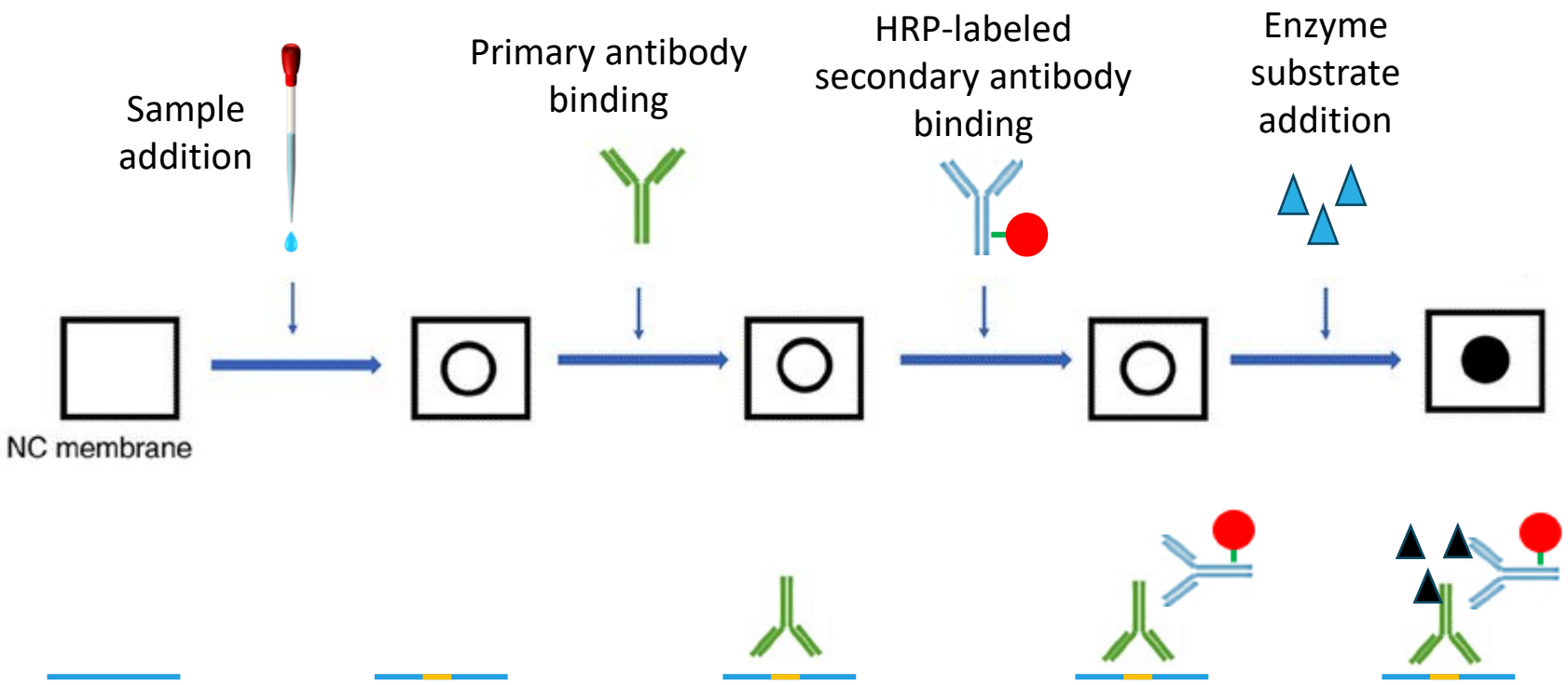
Immunological Assays

dsRNA specific antibodies

Antibody	Isotype & Host	Key Features	Application
J2	Mouse IgG2a	- Highly specific for dsRNA structures (>40 bp) - gold standard	ELISA, IF, FACS, IHC, IP, Dot Blot, ChIP, affinity purification, immunoelectron microscopy
J5	Mouse IgG2b	- Similar affinity and specificity to dsRNA as J2 but with a different isotype	ELISA, dsRNA immunoblotting, immunofluorescence microscopy
K1	Mouse IgG (commonly IgG2a)	- Engineered to overcome limitations of earlier clones - Enhanced signal-to-noise ratio	ELISA, IF, FACS, IHC, IP, Dot Blot, ChIP, affinity purification, immunoelectron microscopy
K2	Mouse IgM	- Isotype alternative to J2/K1 - High Avidity	Sandwich-ELISA, IHC, Dot Blot
1D3	Mouse IgG1	- Recombinant monoclonal	ELISA, IHC, Western Blot

Immunological Assays

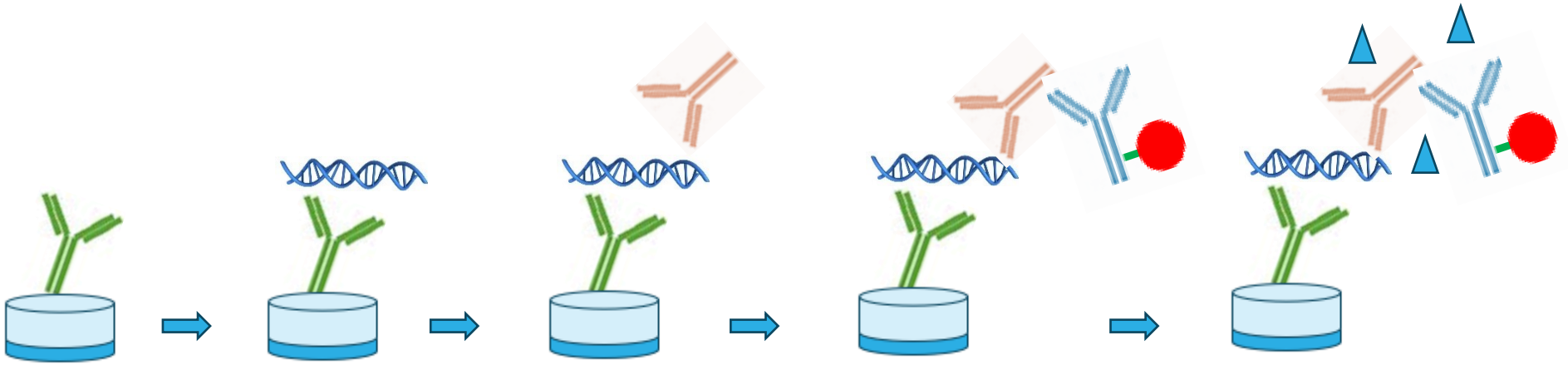
Dot Blot Assay



- **Membrane probed with dsRNA-specific antibodies**
- **Provides qualitative and semi-quantitative results**

Immunological Assays

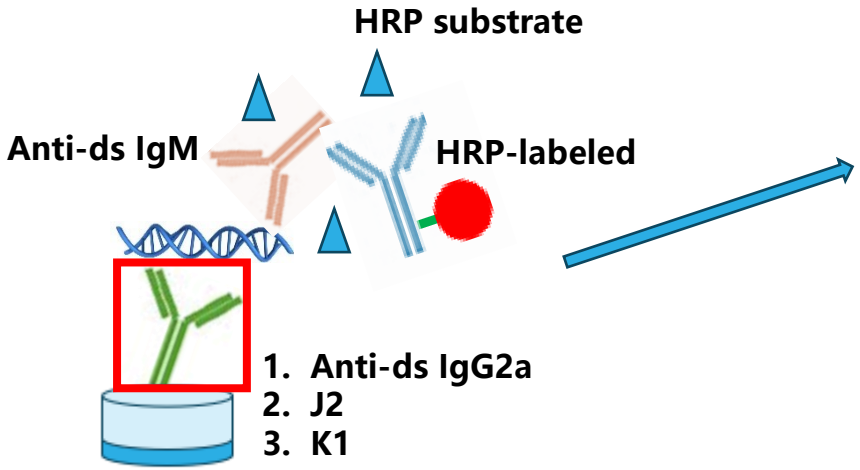
Sandwich ELISA Assay

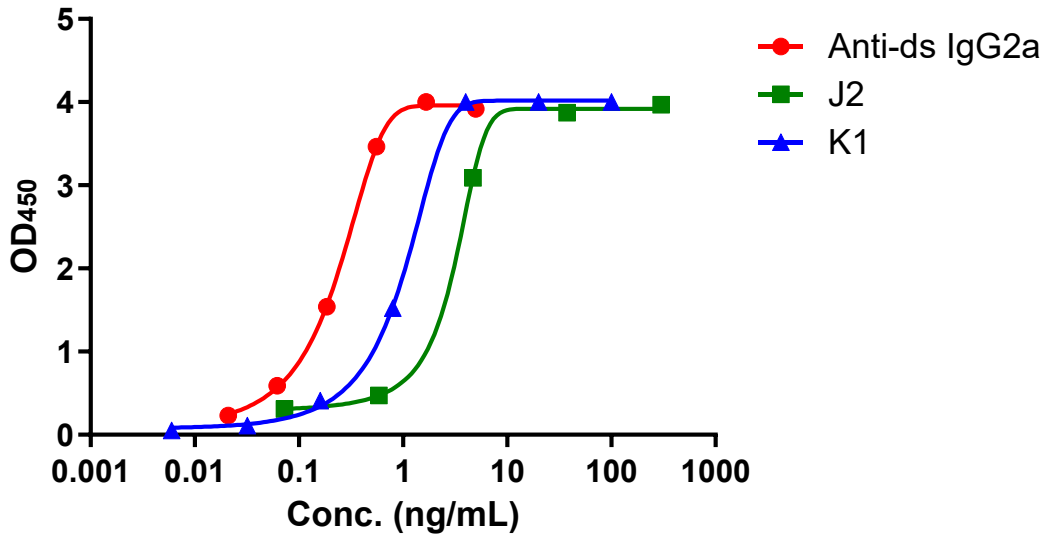


- **dsRNA-specific antibodies in microplate format**
- **High throughput**
- **Quantitative results, LOD can reach < 0.1 ng/mL**

Evaluation of Capture Antibody

Different Capture Antibody



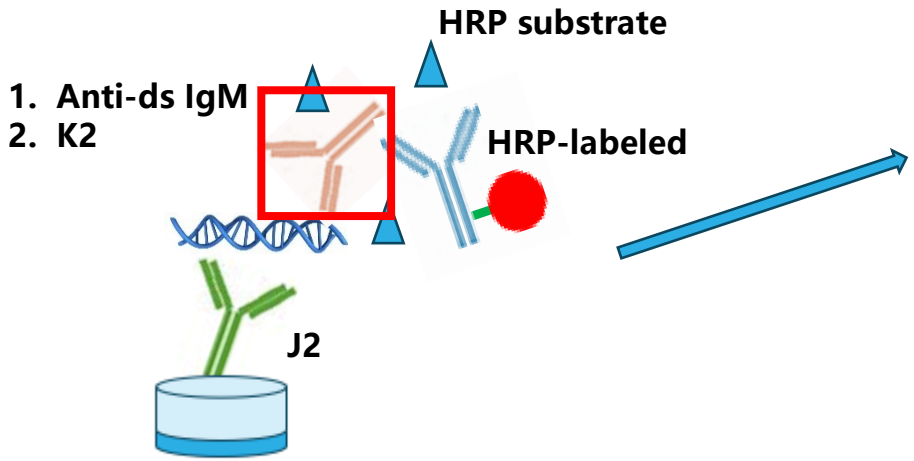


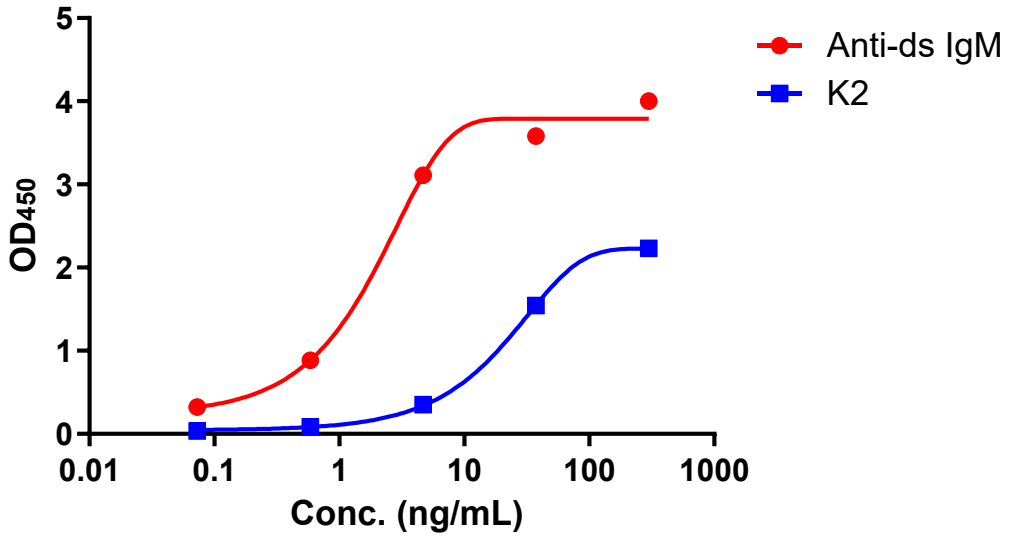
Capture antibody	EC_{50} (ng/mL)
Anti-ds IgG2a	0.25
J2	2.60
K1	1.00

Detection antibody: Anti-ds IgM antibody
Sample: dsRNA 500 bp (Uridine)

Evaluation of Detection Antibody

Different Detection Antibody



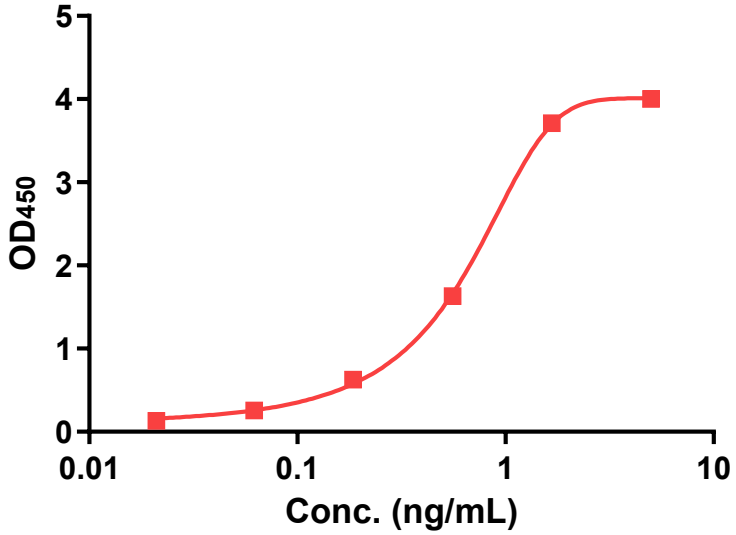


Detection antibody	EC_{50} (ng/mL)
Anti-ds IgM	1.09
K2	22.17

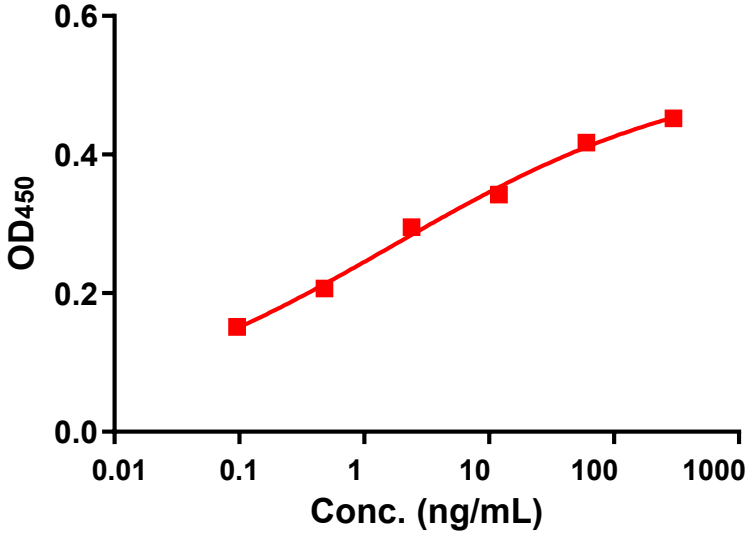
Capture antibody: J2 antibody
Sample: dsRNA 500 bp (Uridine)

Evaluation of Antibody Pairs

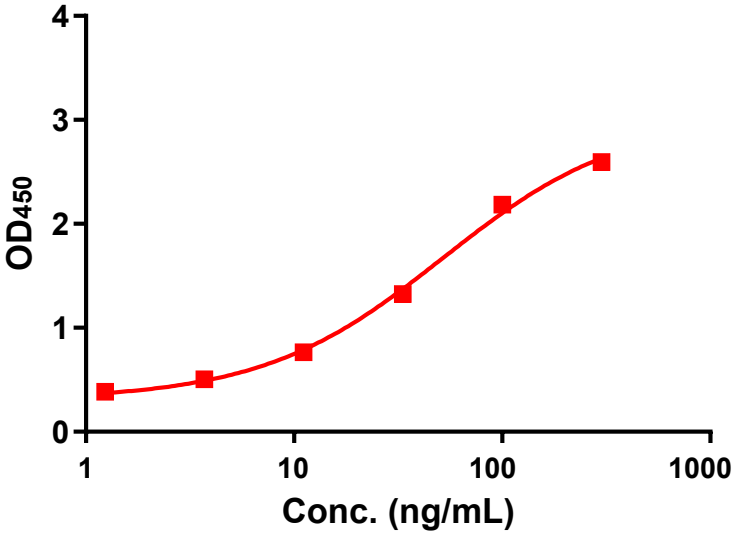
Anti-ds IgG2a-Anti-ds IgM



K1-K2



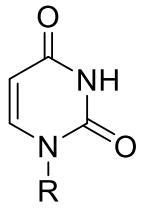
J2-K2



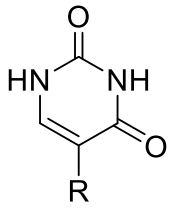
Sample: dsRNA 500bp (Uridine)

Influence of Modified Nucleosides

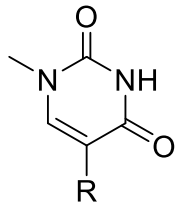
Different Base Modification



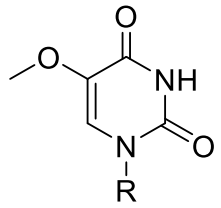
U



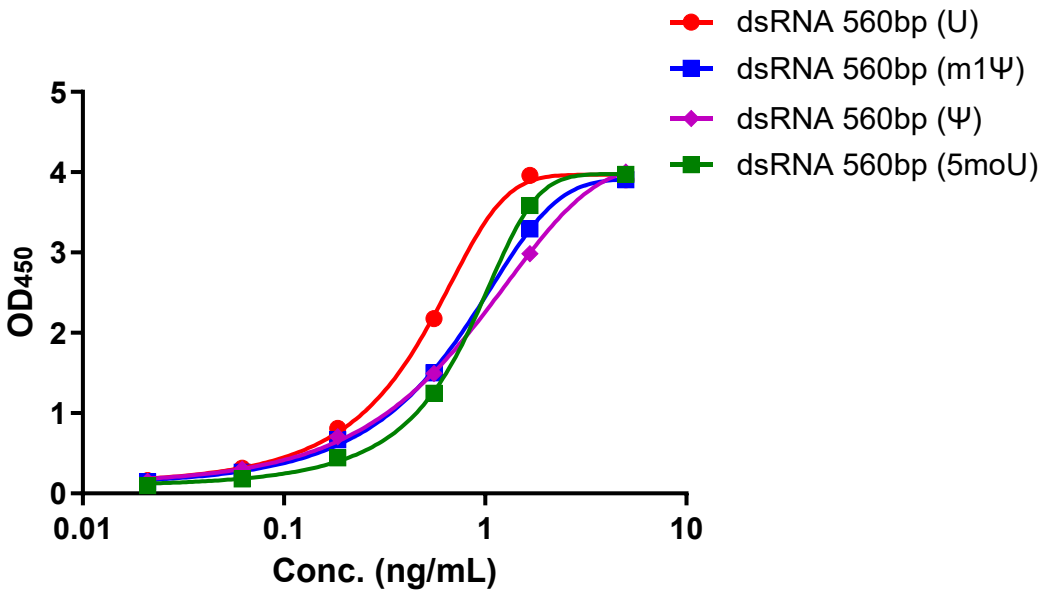
Ψ

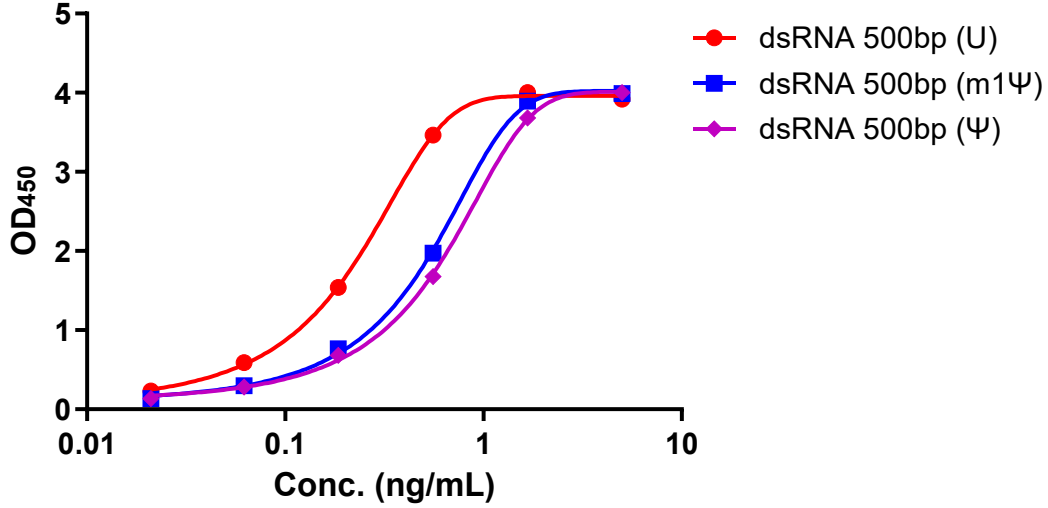


m1Ψ



5moU

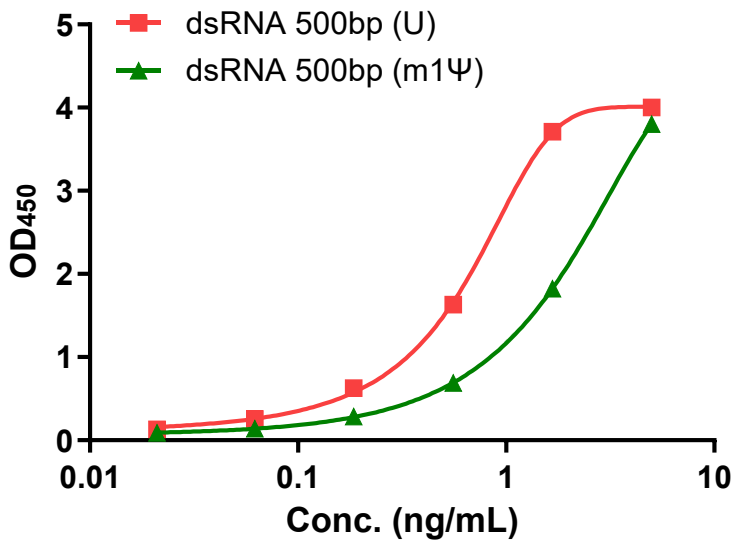




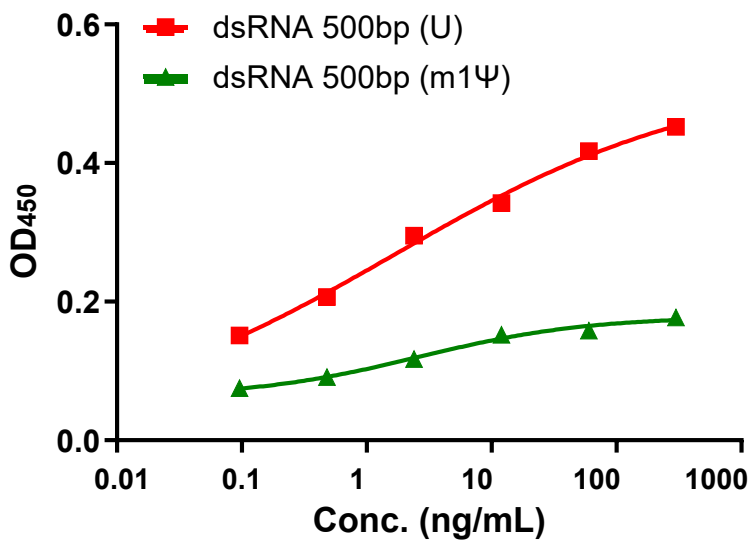
Influence of Base Modification

Different Antibody Pairs

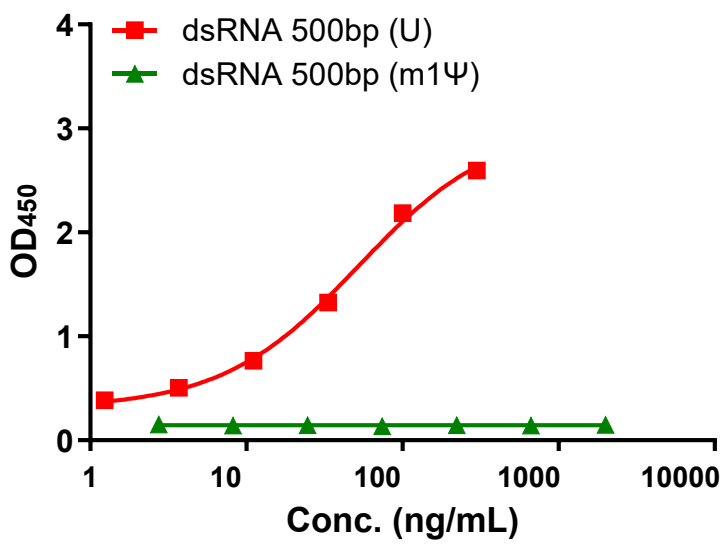
Anti-ds IgG2a-Anti-ds IgM



K1-K2

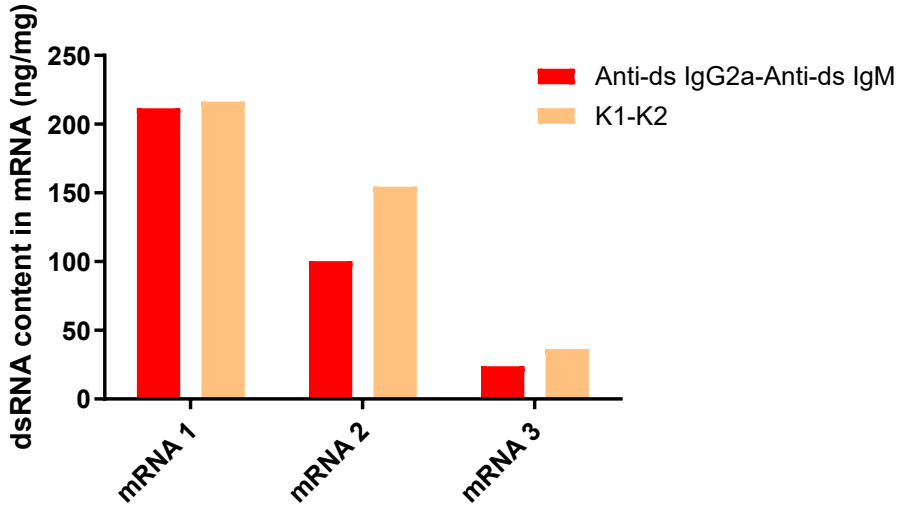


J2-K2



dsRNA content in IVT mRNA

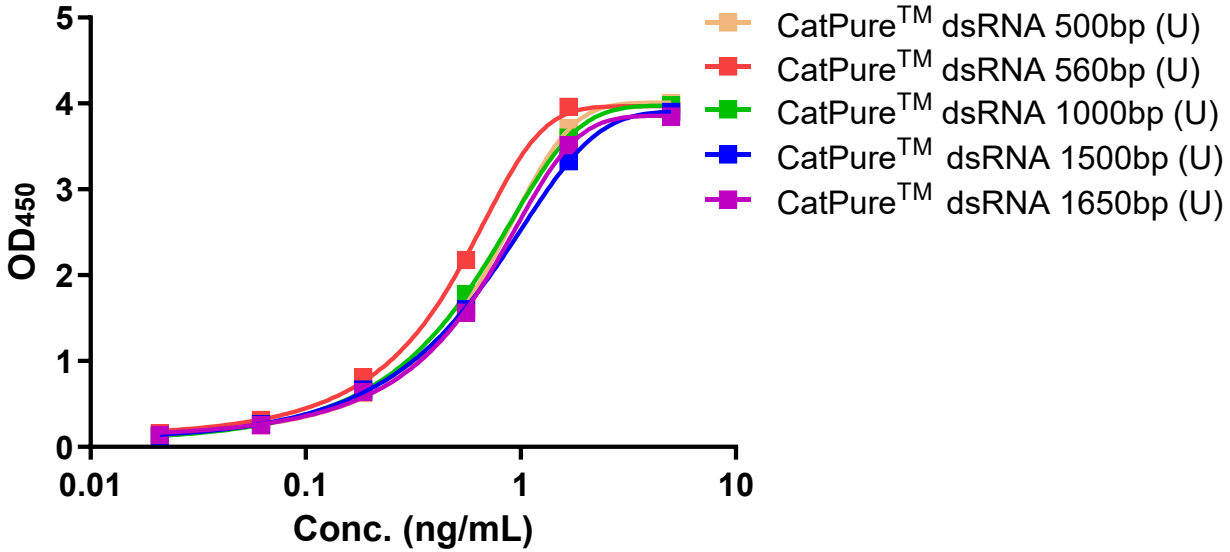
Different Antibody Pairs



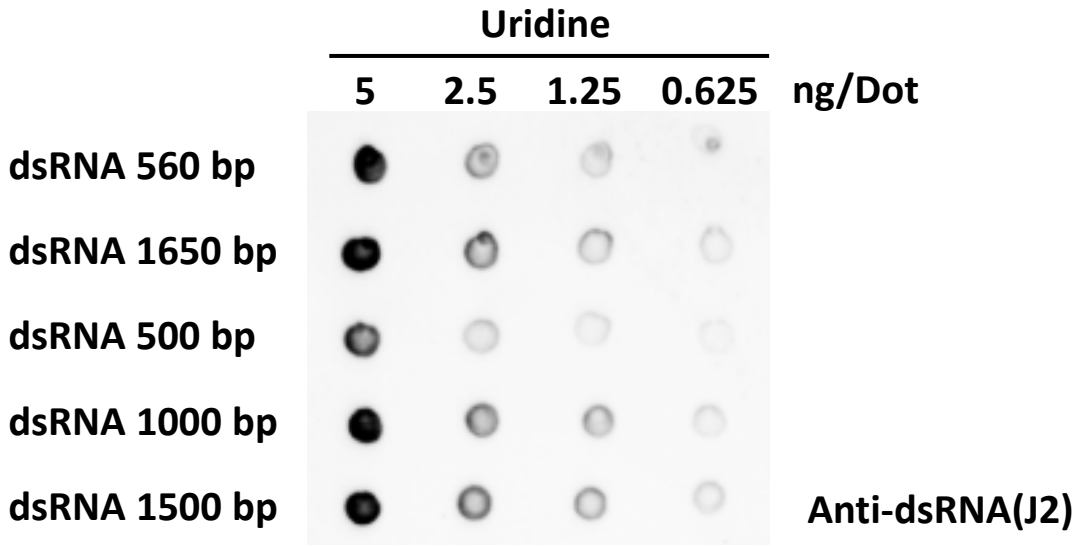
Sample	Base Modification	dsRNA (ng/mg)	dsRNA (ng/mg)
		Anti-ds IgG2a-Anti-ds IgM	K1-K2
mRNA 1	m1Ψ	211.6	216.3
mRNA 2	m1Ψ	100.4	154.5
mRNA 3	m1Ψ	23.8	36.4

➢ **Relatively consistent results**

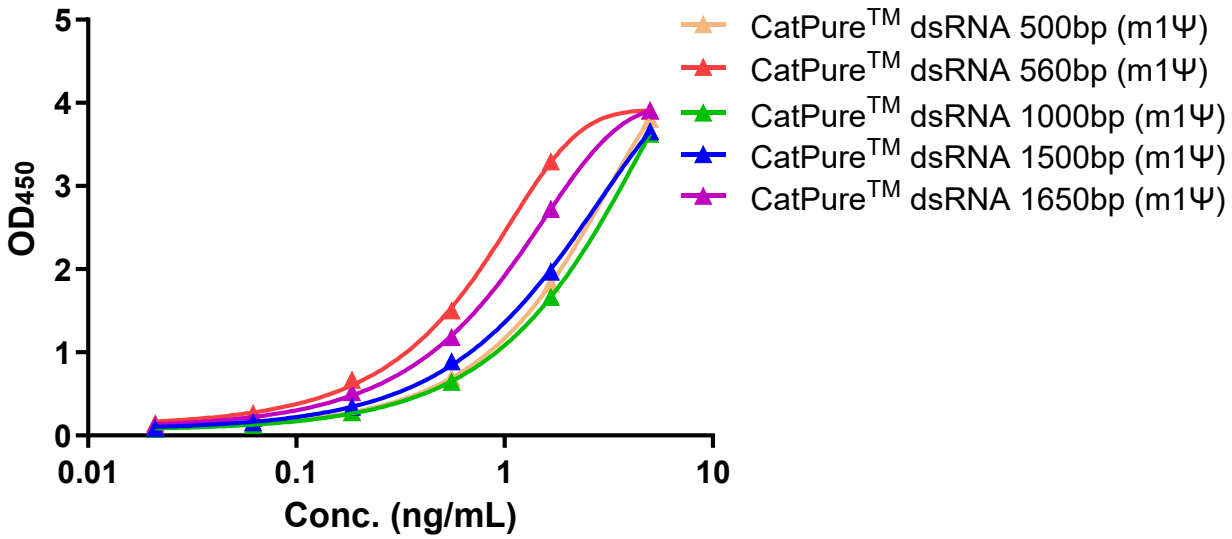
dsRNA Length



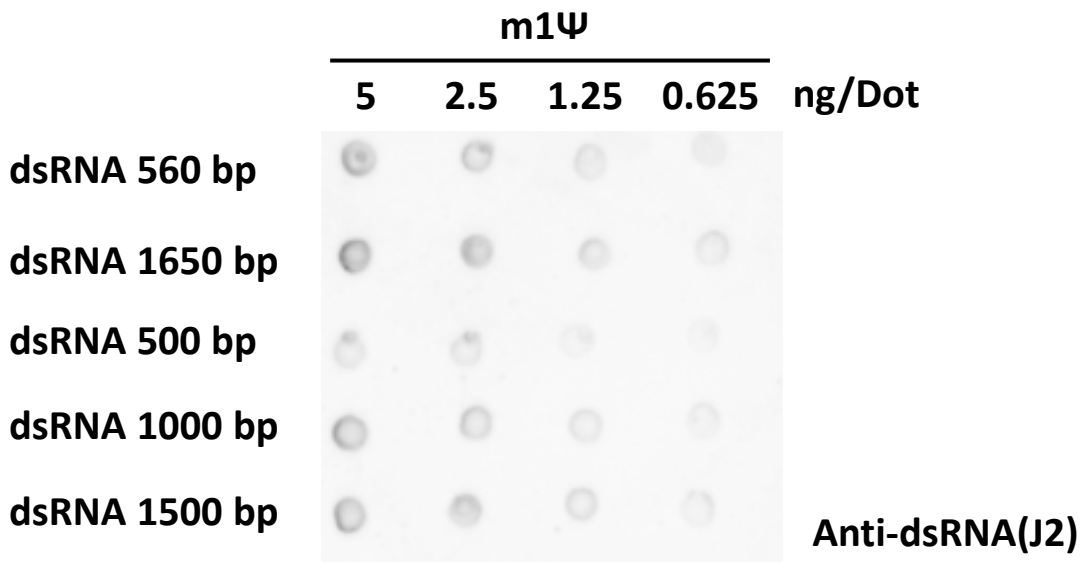
Sample	EC50 (ng/mL)
dsRNA 500bp (U)	0.70
dsRNA 560bp (U)	0.52
dsRNA 1000bp (U)	0.66
dsRNA 1500bp (U)	0.75
dsRNA 1650bp (U)	0.71



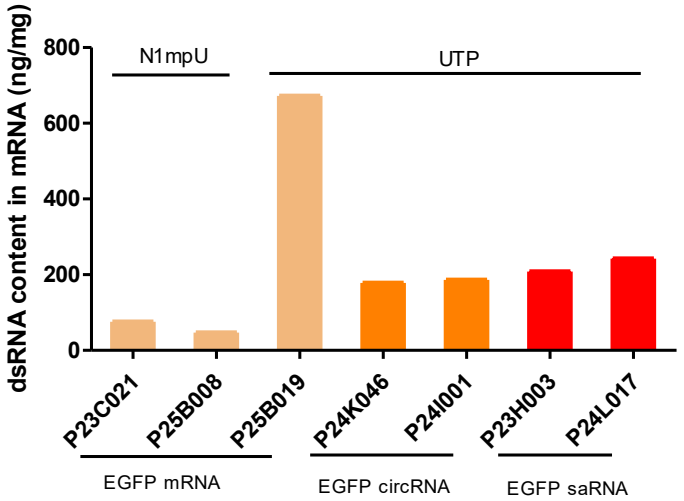
dsRNA Length

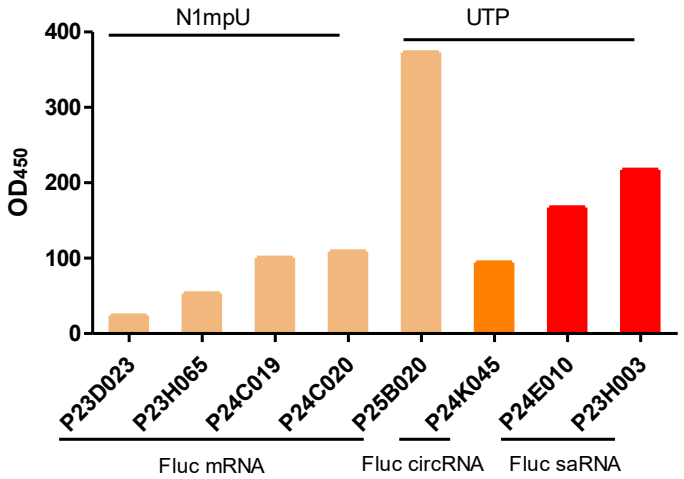


Sample	EC50 (ng/mL)
dsRNA 500bp (m1Ψ)	4.51
dsRNA 560bp (m1Ψ)	0.80
dsRNA 1000bp (m1Ψ)	7.12
dsRNA 1500bp (m1Ψ)	4.52
dsRNA 1650bp (m1Ψ)	1.31



RNA Modalities

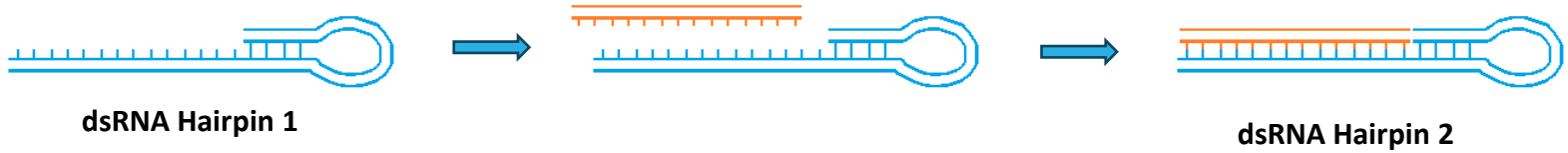


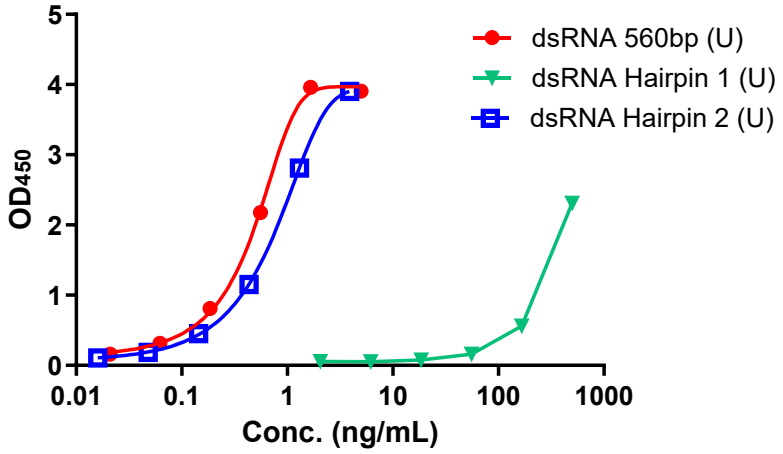


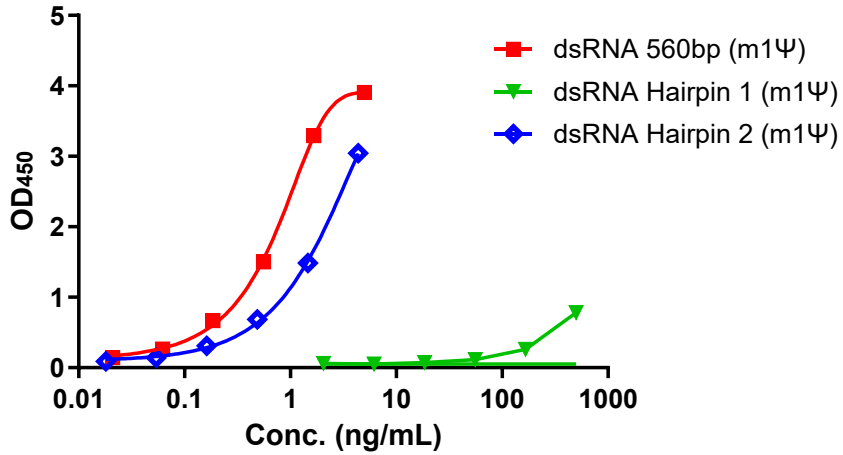
Modality	Encoded Protein	Base Modification	OD_{450} (at 1 ng/μL)	dsRNA (ng/mg)
mRNA	EGFP	U	2.587	672.6
mRNA		m1Ψ	1.240	275.0
mRNA		m1Ψ	0.371	75.9
mRNA		m1Ψ	0.259	47.1
CircRNA		U	1.495	178.0
CircRNA		U	1.557	186.0
SaRNA		U	1.730	208.0
SaRNA		U	1.979	242.0

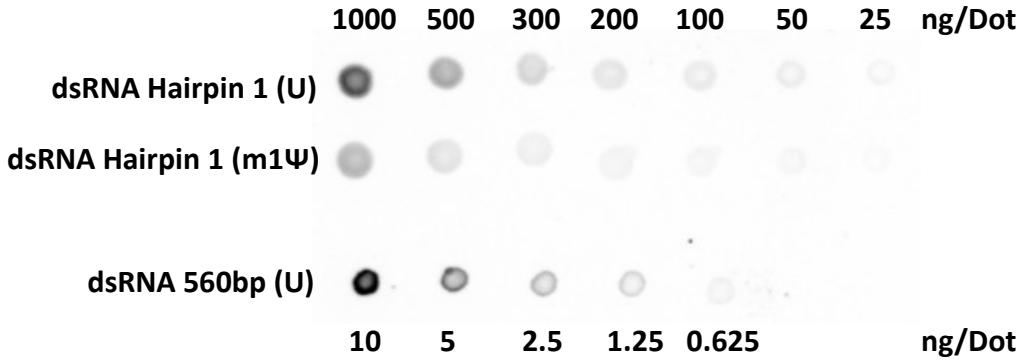
Modality	Encoded Protein	Base Modification	OD_{450} (at 1 ng/μL)	dsRNA (ng/mg)
mRNA	Fluc	U	1.224	372.9
mRNA		m1Ψ	0.485	23.8
mRNA		m1Ψ	0.340	53.0
mRNA		m1Ψ	0.419	100.4
mRNA		m1Ψ	0.433	108.7
CircRNA		U	0.823	94.0
SaRNA		U	1.407	167.0
SaRNA		U	1.797	217.0

dsRNA with Hairpin





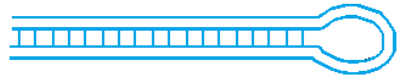




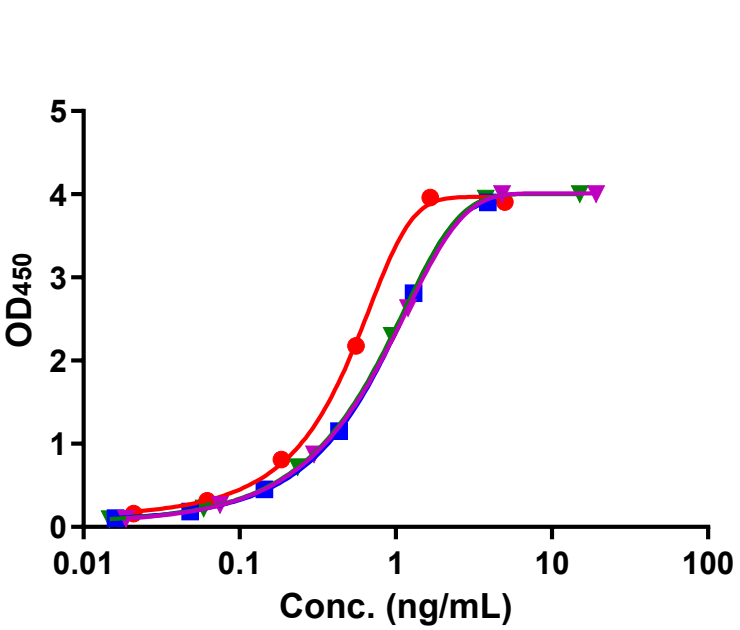
Sample	Length	Theoretical dsRNA content	Theoretical dsRNA content	Observed dsRNA content
dsRNA Hairpin 1 (U)	~880 nt	70 bp	15.8 %	0.1 %
dsRNA Hairpin 1 (m1Ψ)	~880 nt	70 bp	15.8%	< 0.1 %

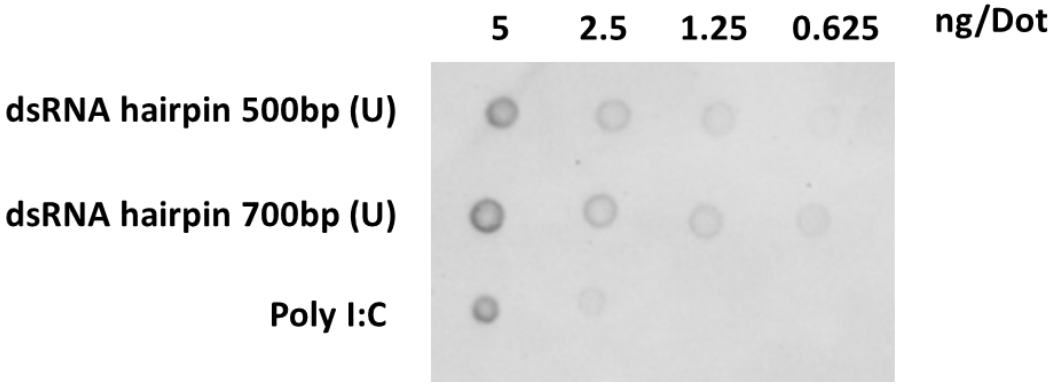
dsRNA with Hairpin

dsRNA Hairpin 2



dsRNA Hairpin 500bp &
dsRNA Hairpin 700bp





Thanks You!

Q&A