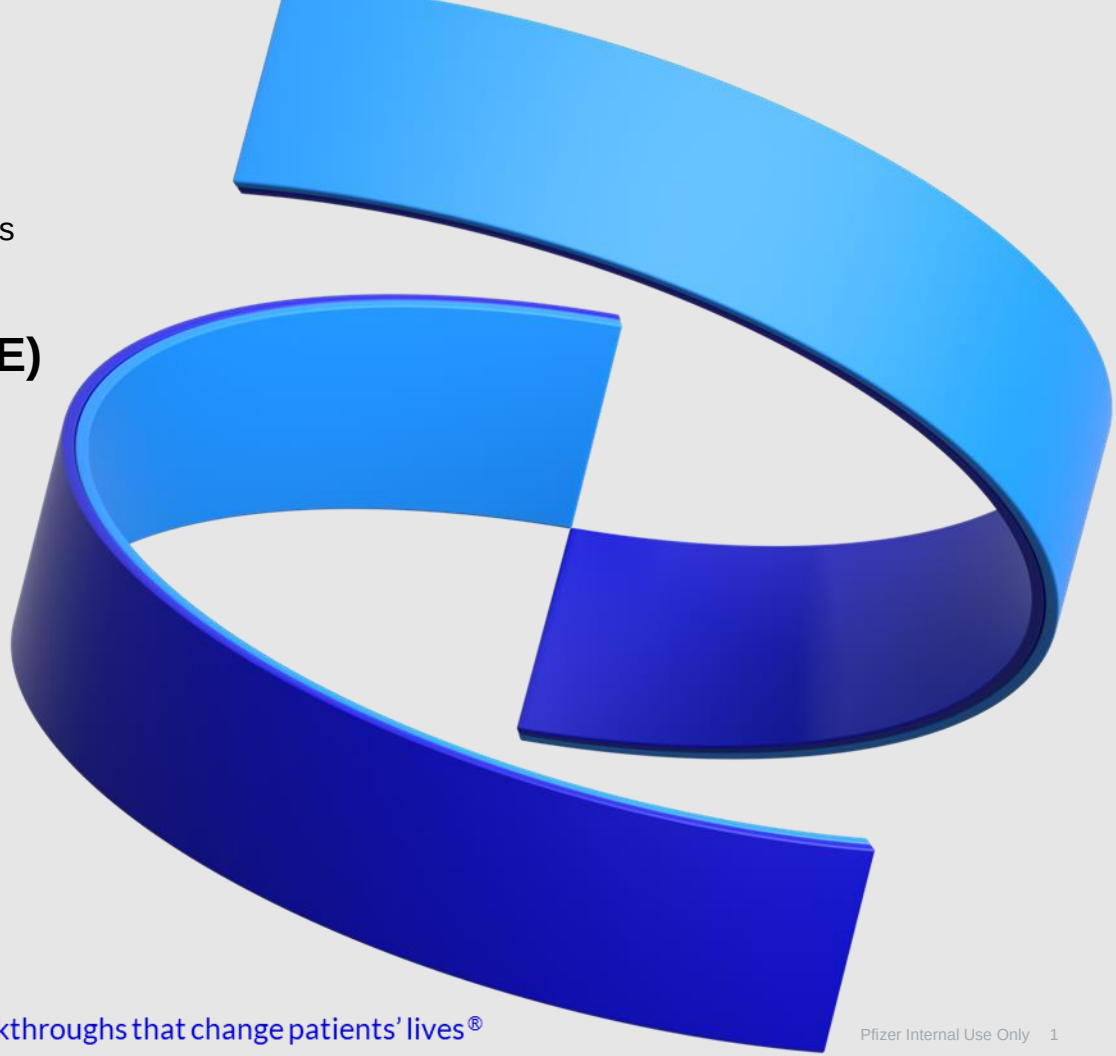
BIOASSAYS 2023

Scientific Approaches and Regulatory Strategies

In-vitro Expression Assay (IVE) for potency expression of mRNA vaccine product



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***Pfizer*, VRD**

Topics Overview

- Introduction to modern potency assay techniques and new vaccine technology in an established space
- Approaches and challenges to “platform” release methods for quick validation of new strains
- Lessons from rapid pandemic response for other programs
- Correlation of *in-vitro* and *in-vivo* data (*in-vitro* expression as reflection of immunogenicity)

Considerations When Establishing A Potency Assay for mRNA Vaccine Products

In-vitro

- Faster
- Cheaper
- Safer
- Measures drug product itself directly
- Less variable
- Much greater correlation value
- “Platform-ability” to test various antigens

- More limited view of immune system response

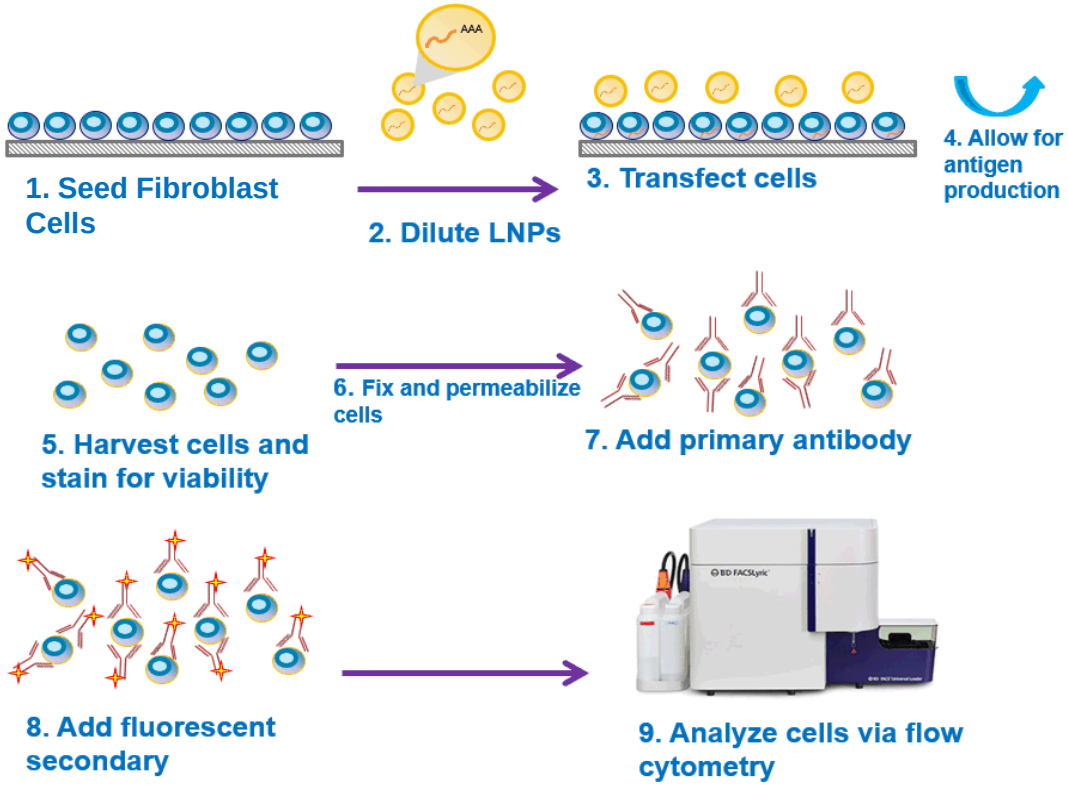
In-vivo

- More comprehensive view of the immune system response

- Takes longer
- Much more expensive
- More variability

Overview of the *In-vitro* Expression (IVE) Assay

Confirm protein *in-vitro* expression by flow cytometry



Main Considerations in Developing an IVE Potency Method

Antibody Selection

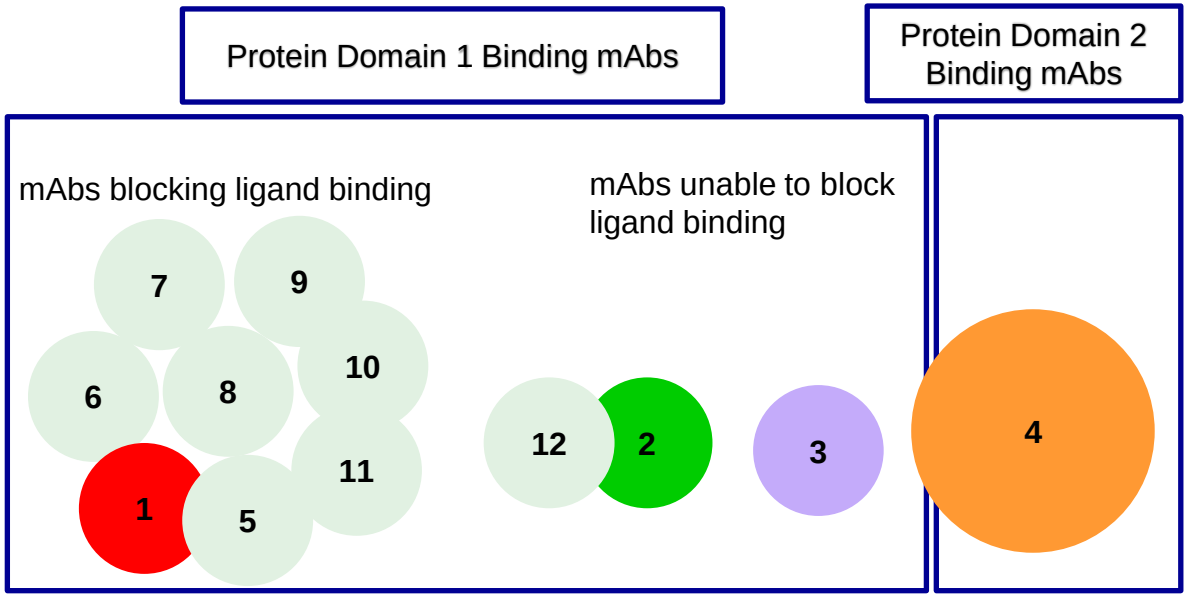
- Screen of Available Commercial/ In-house Antibodies
- Neutralizing Antibody, if available
- Monoclonal vs. Polyclonal
- Host species
- Epitope mapping (viral strain specificity, mutations)

Cell Line

- Permissible to transfectability and antigen expression
- Immune competency
- Ease of handling and maintenance
- Fast growth rate for quick test turnaround time

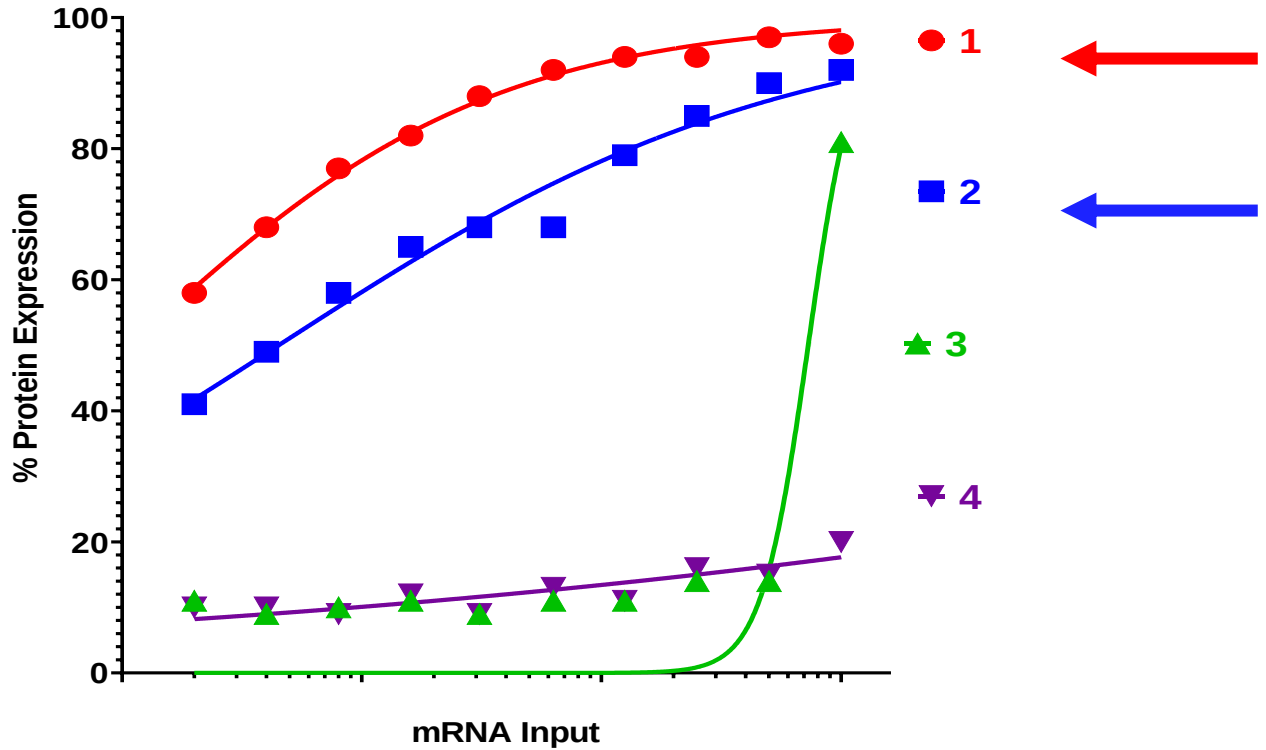
Selection of Neutralizing mAbs that Bind to Four Non-overlapping Epitopes

Primary Antibodies – Protein X		
Item	**Source**	**Epitope Bins**
1	In-House	Bin-1
2	In-House	Bin-2
3	In-House	Bin3
4	In-House	Bin-4

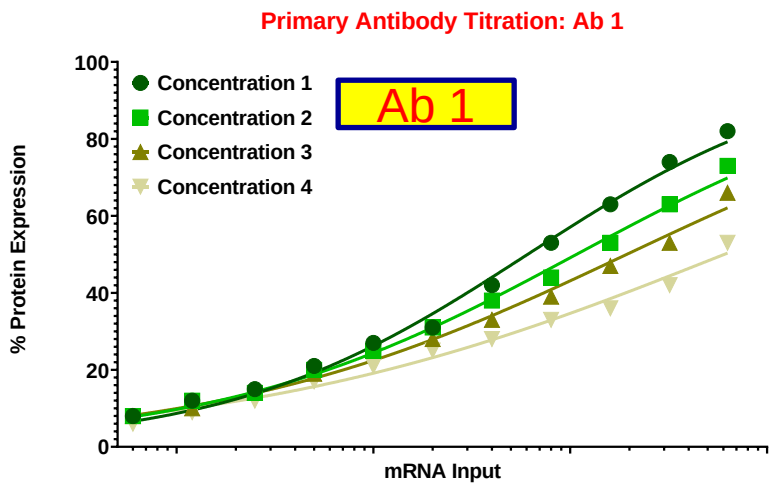


Epitope	Bin-1	Bin-2	Bin-3	Bin-4

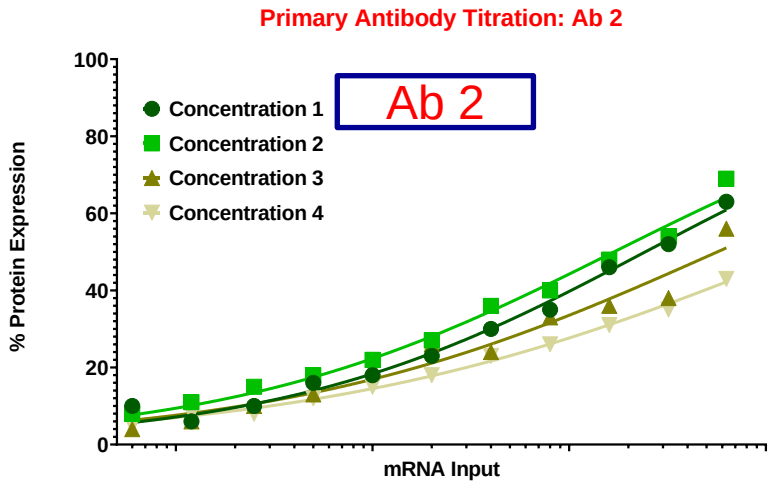
Initial Antibody Screening Shows Antibody 1 and 2 Outperforming the Rest



Screening of Top 2 In-house Antibodies for IVE Assay Highlights Antibody 1 as Best Choice

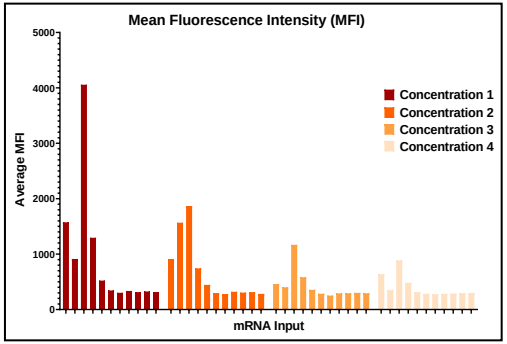


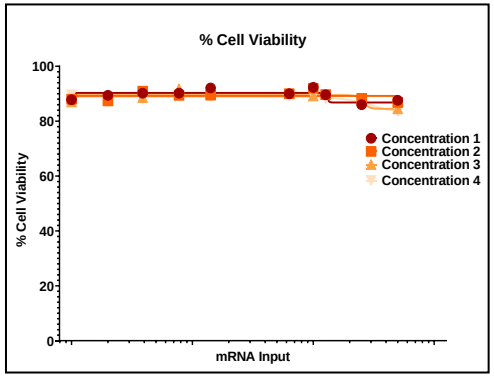
Antibody:	Primary Antibody Concentration	Secondary Antibody Concentration	% Protein Expression	EC50 (unit/well)
Ab 1	1	1	82	6
	2		73	11
	3		66	19
	4		53	61

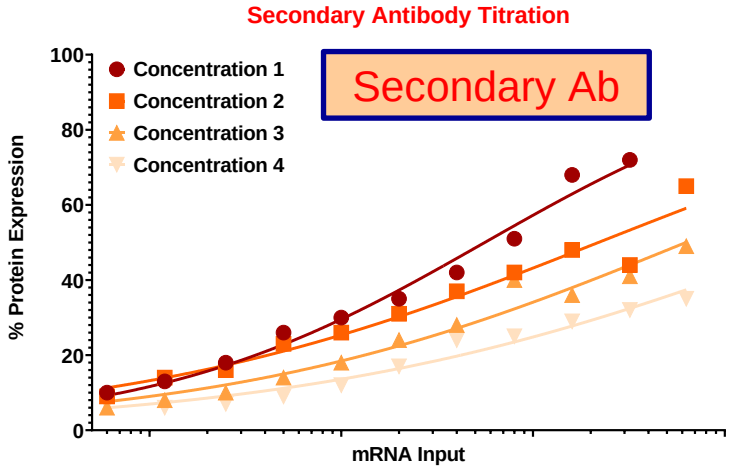


Antibody:	Primary Antibody Concentration	Secondary Antibody Concentration	% Protein Expression	EC50 (unit/well)
Ab 2	1	1	63	24
	2		69	17
	3		56	57
	4		43	153

Mean Fluorescence Intensity (MFI) Data Support % Protein Expression Trends With High Cell Viability

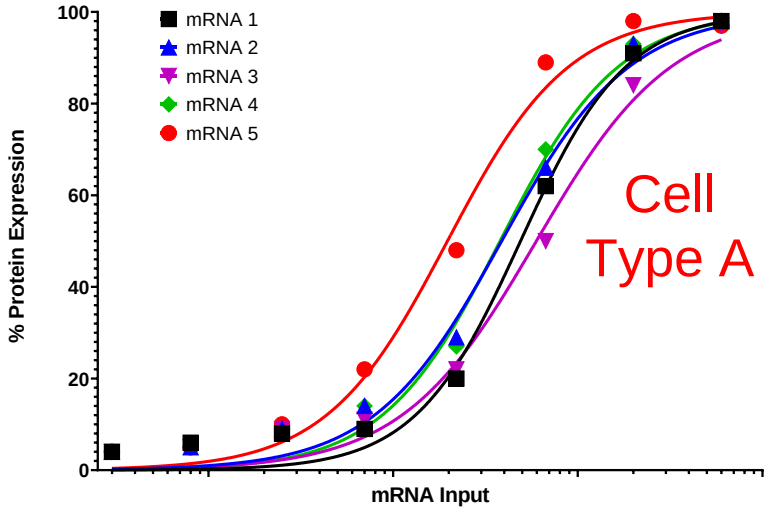


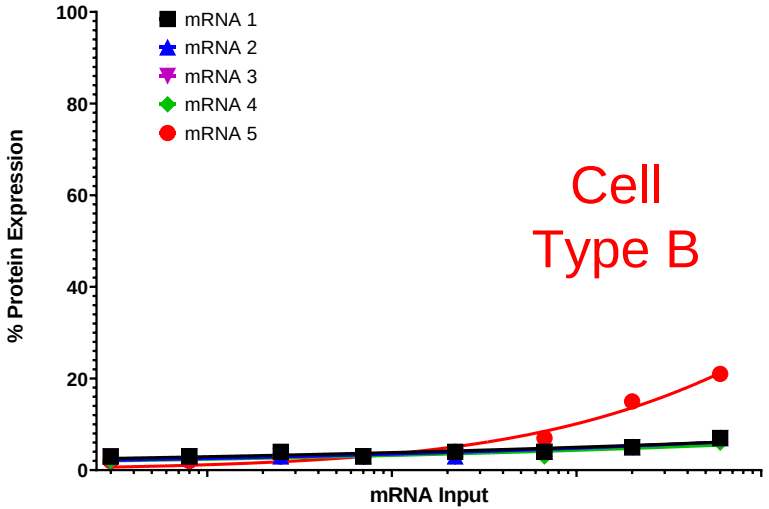




Primary Antibody Concentration	Secondary Antibody Concentration	% Protein Expression	EC50 (unit/well)
1	1	72	6
	2	65	22
	3	49	63
	4	35	311

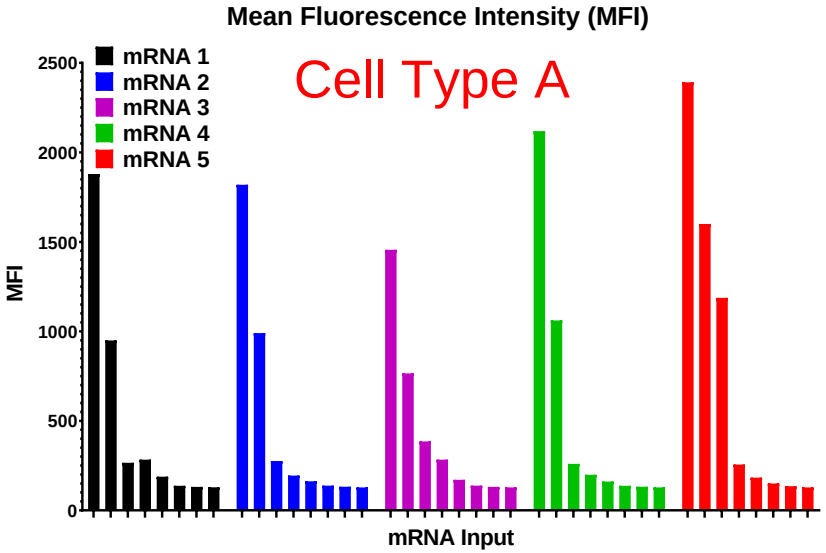
Choice of Cells for the IVE Assay Matters

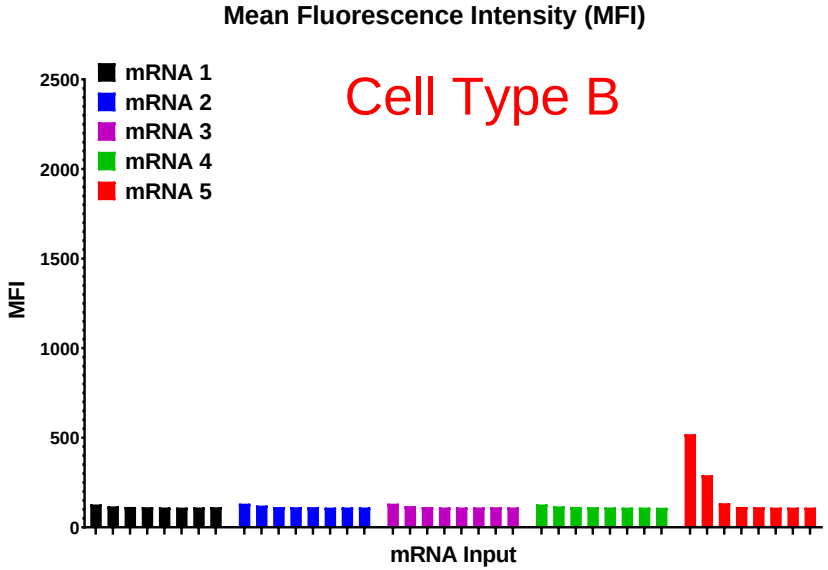




	Cell Type A		Cell Type B	
Construct	% Protein Expression	EC50 (unit/well)	% Protein Expression	EC50 (unit/well)
mRNA 1	91	49	5	>top dose of curve
mRNA 2	93	39	5	>top dose of curve
mRNA 3	84	60	5	>top dose of curve
mRNA 4	93	38	5	>top dose of curve
mRNA 5	98	20	15	>top dose of curve

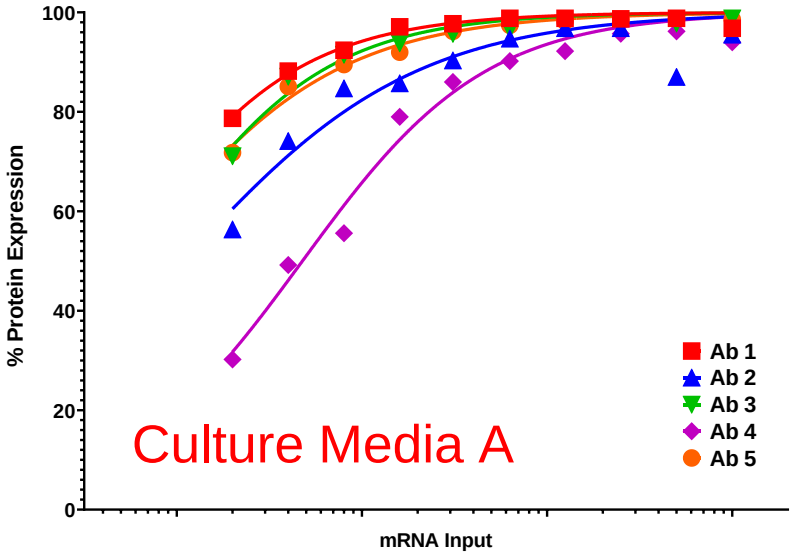
Choice of Cells for the IVE Assay Matters

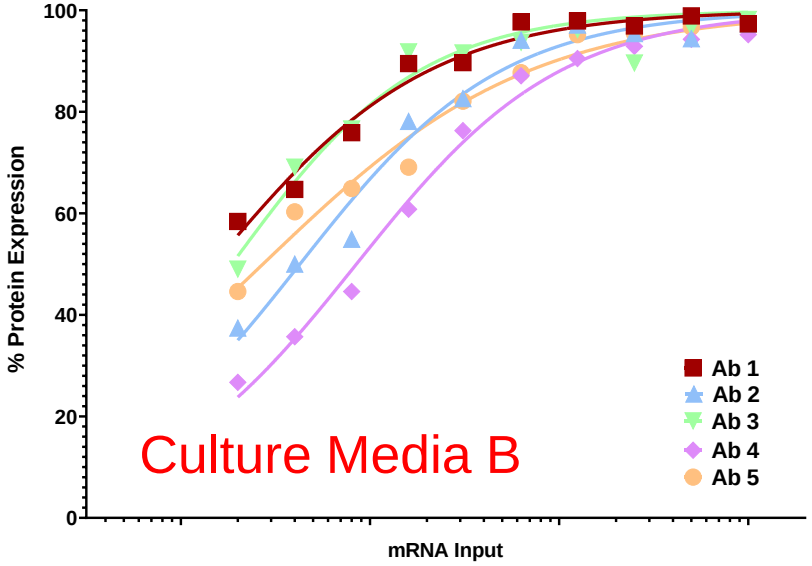




	Cell Type A		Cell Type B	
Construct	% Protein Expression	MFI	% Protein Expression	MFI
mRNA 1	91	949	5	117
mRNA 2	93	990	5	121
mRNA 3	84	765	5	118
mRNA 4	93	1061	5	117
mRNA 5	98	1600	15	291

Media Composition May Impact Protein Expression

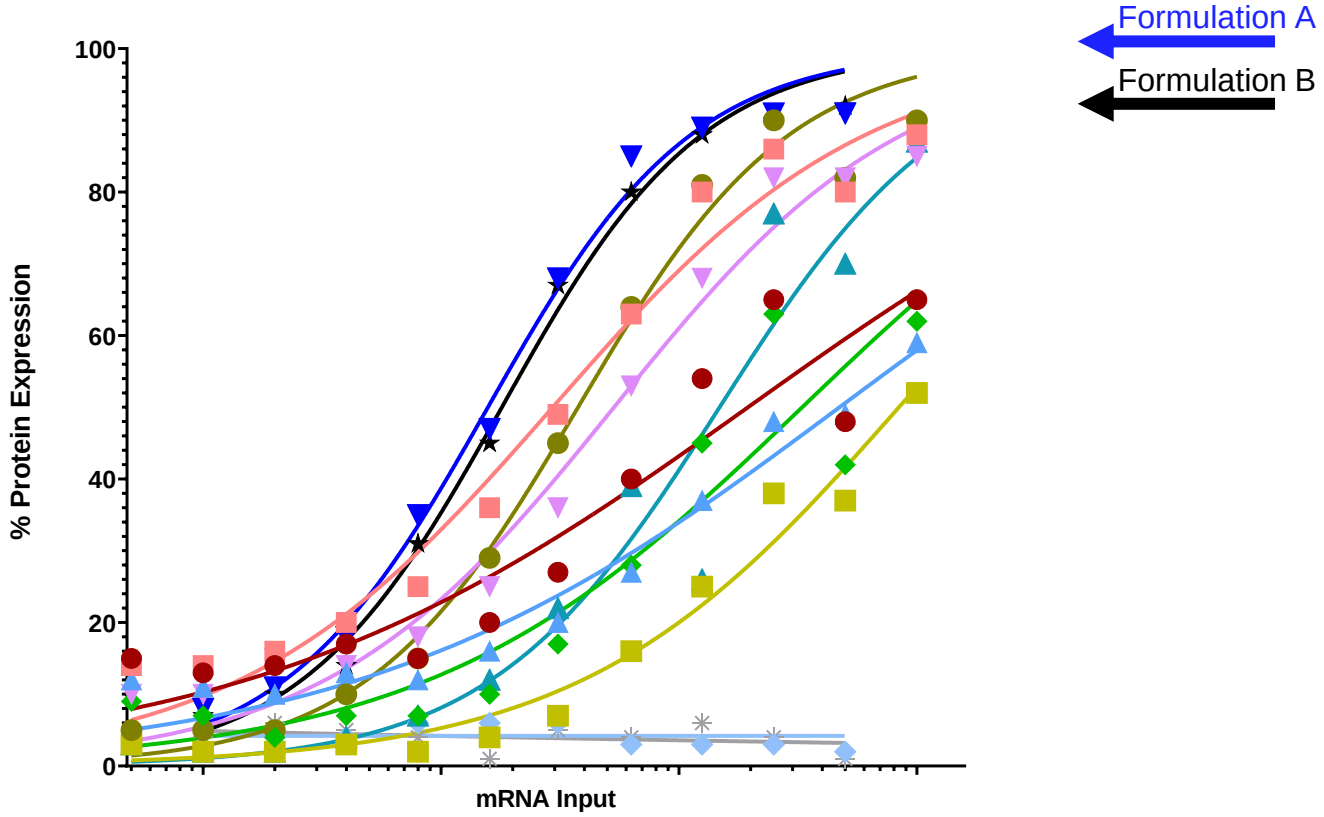




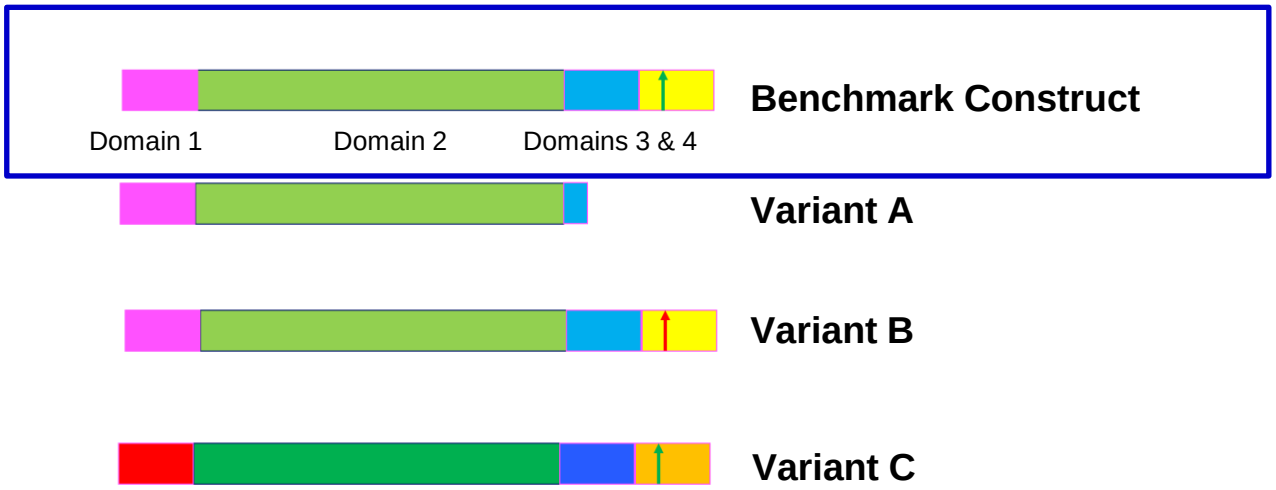
Antibody	% Protein Expression	EC50 (unit/well)
Ab 1	97	0.5
Ab 2	86	1.1
Ab 3	94	0.7
Ab 4	79	4.8
Ab 5	92	0.6

Antibody	% Protein Expression	EC50 (unit/well)
Ab 1	90	2
Ab 2	78	4
Ab 3	92	2
Ab 4	61	9
Ab 5	69	3

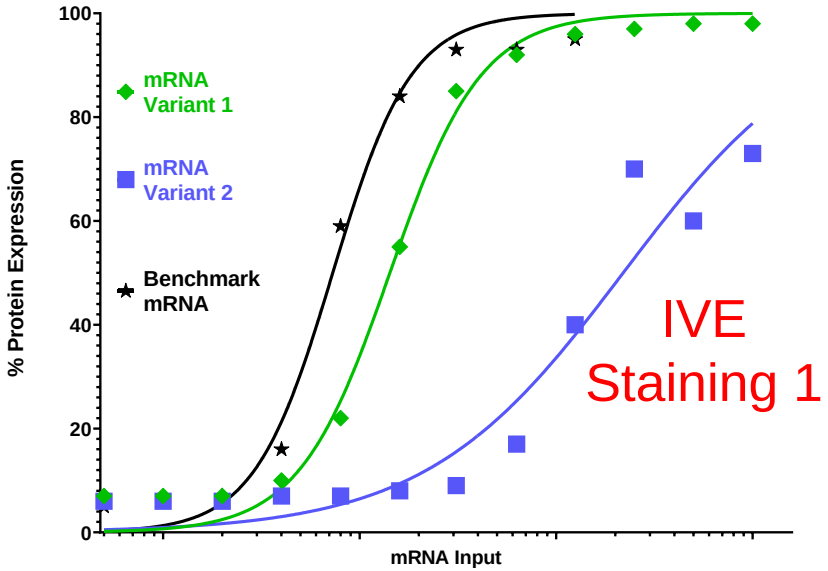
Potency Expression Screen by the IVE Assay Highlights mRNA Products with Formulations A and B as Best Performers

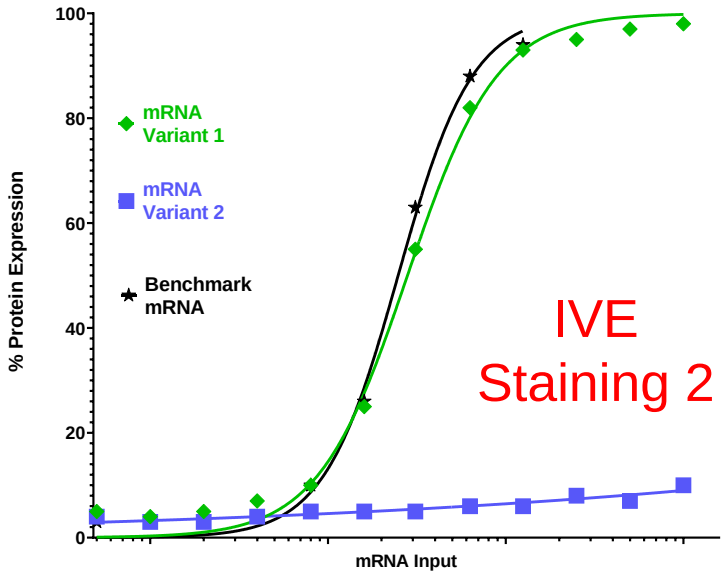


IVE Assay is a Potent Tool to Screen for Potency Expression Differences between mRNA Vaccine Variants and Benchmark Constructs



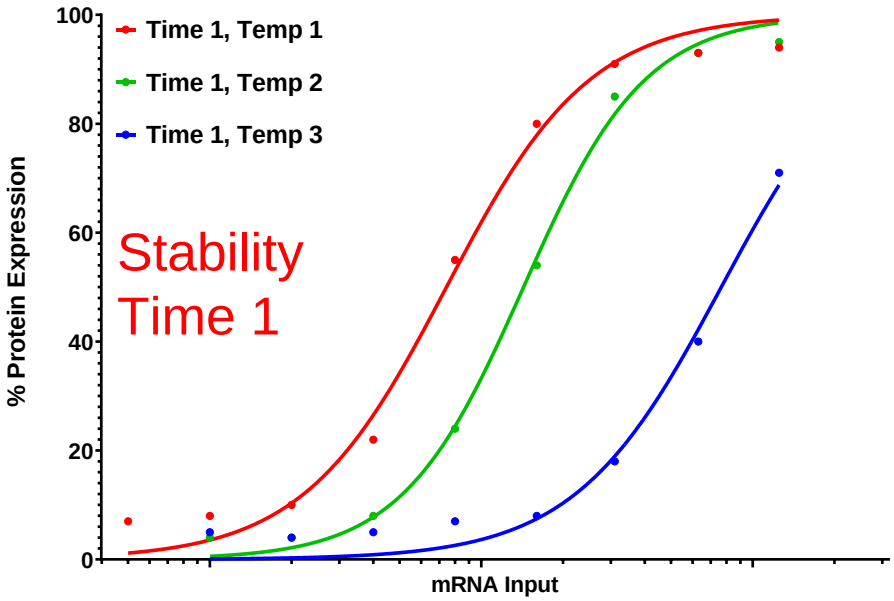
mRNA Variant 2 Underperforms Two Other Constructs Regardless of Cellular Localization

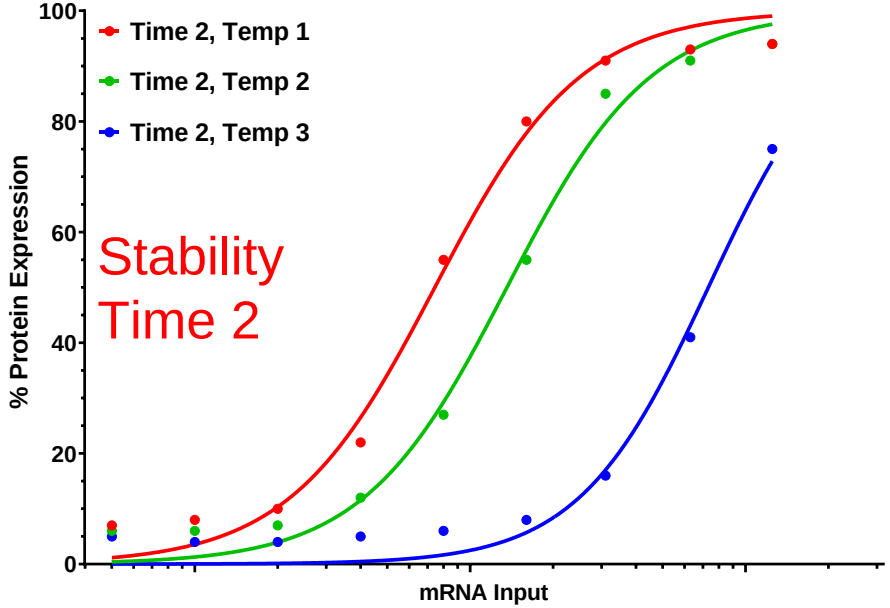




Construct	IVE Staining 1			IVE Staining 2		
	EC50	% Positive Cells	MFI	EC50	%Positive Cells	MFI
mRNA Variant 1	14	85	543	28	55	532
mRNA Variant 2	220	9	162	<LOQ	5	262
Benchmark mRNA	7	93	1159	25	63	622

Potency Expression of mRNA Vaccine Products Remains Stable Over Time at All Temperatures





IVE Potency Summary

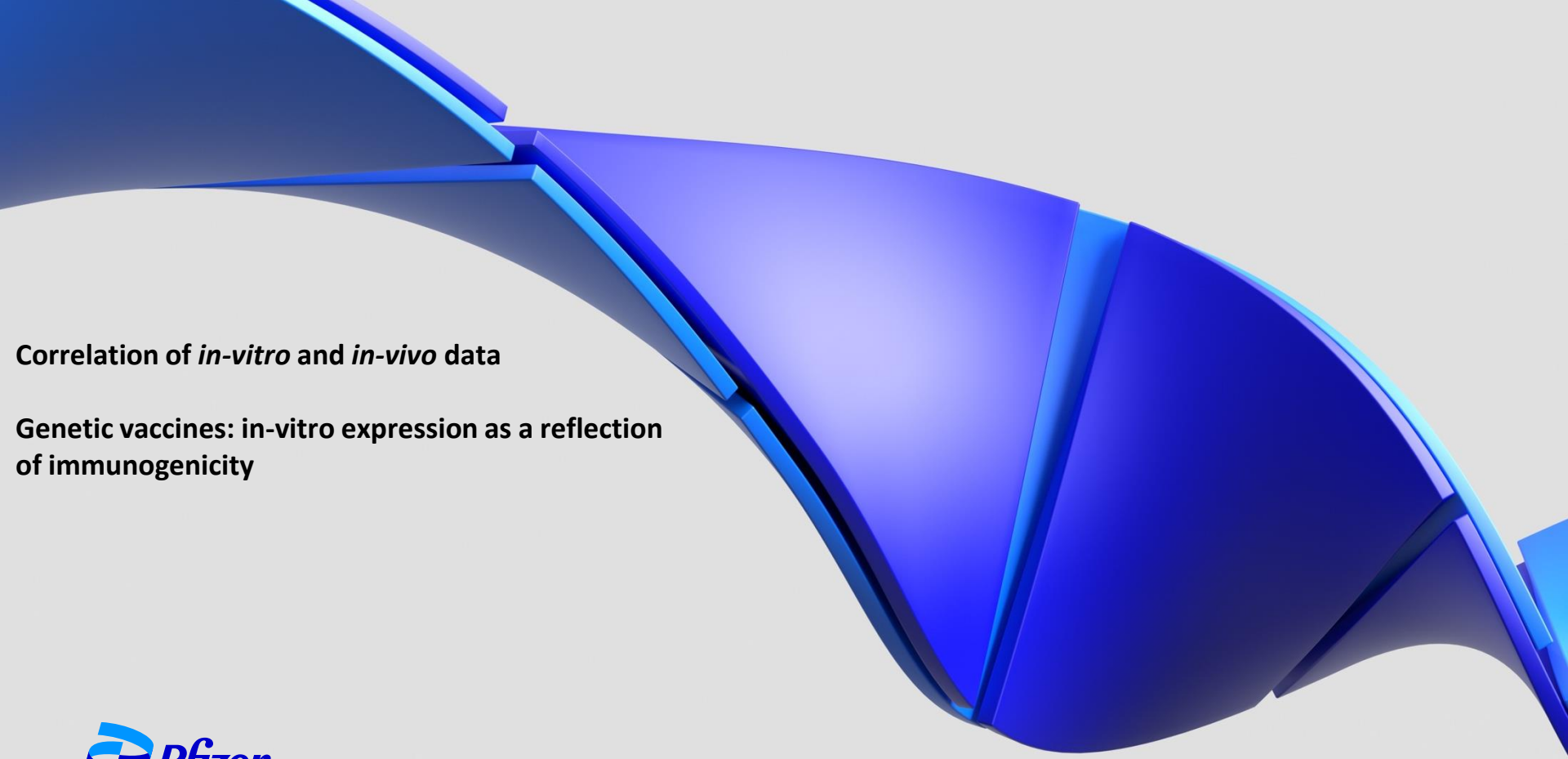
Conclusions:

Assay Establishment:

- Appropriate cell type selection is critical for a robust assay
- Screening and optimization of antibodies for performance in the assay are critical steps
- Ensure that cells are viable and fluorescence intensity shows a strong signal
- Expression is influenced by media conditions

Assay Applications:

- Assay is “platform-able” to allow for testing of various antigens and construct designs
- Assay is stability indicating to monitor loss of potency at different storage temperatures over time



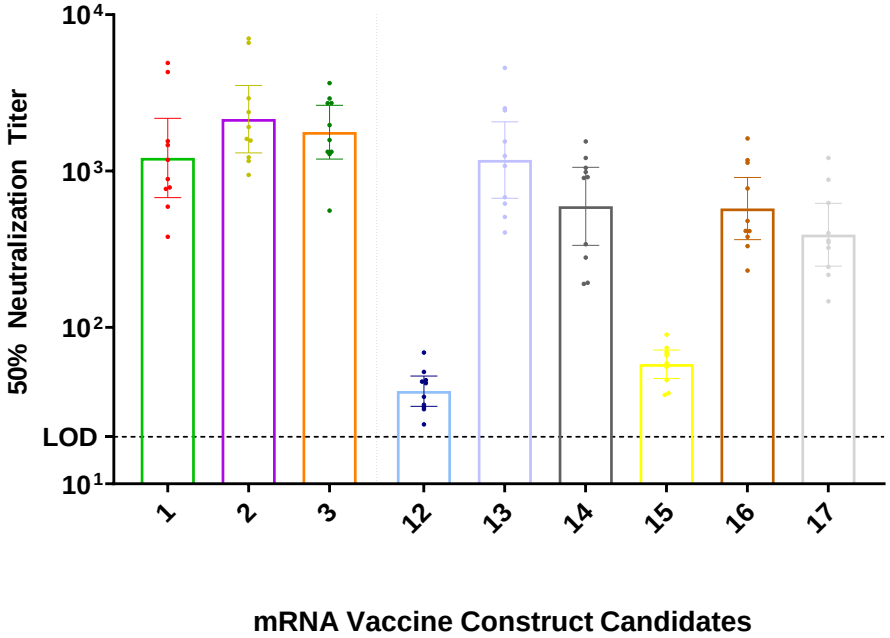
Correlation of *in-vitro* and *in-vivo* data

Genetic vaccines: in-vitro expression as a reflection of immunogenicity

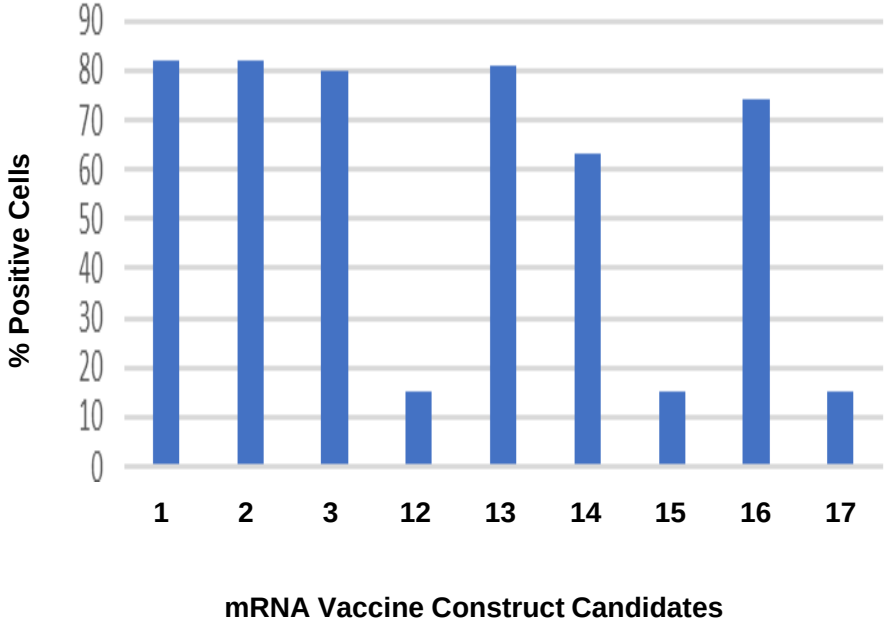
Pfizer
Worldwide Research, Development and Medical
Vaccine Research and Development

In-vitro Expression is Predictive of *in-vivo* Performance

In-vivo Data

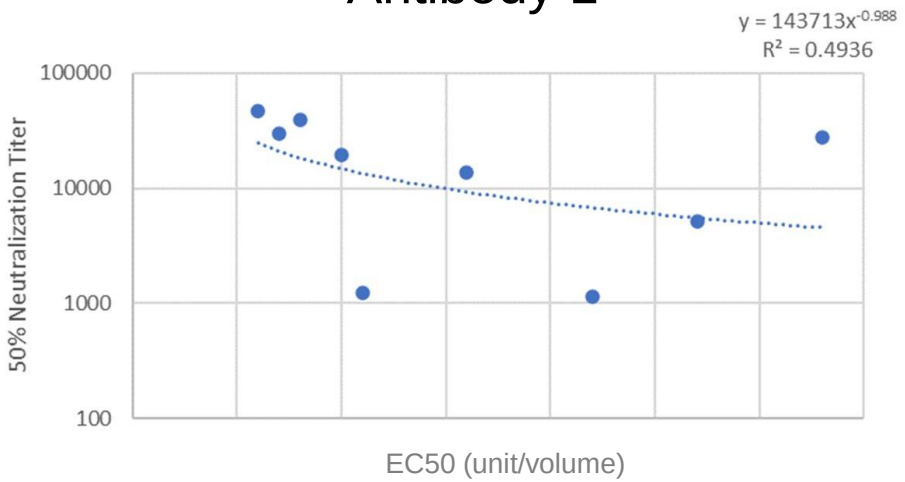


In-vitro Data

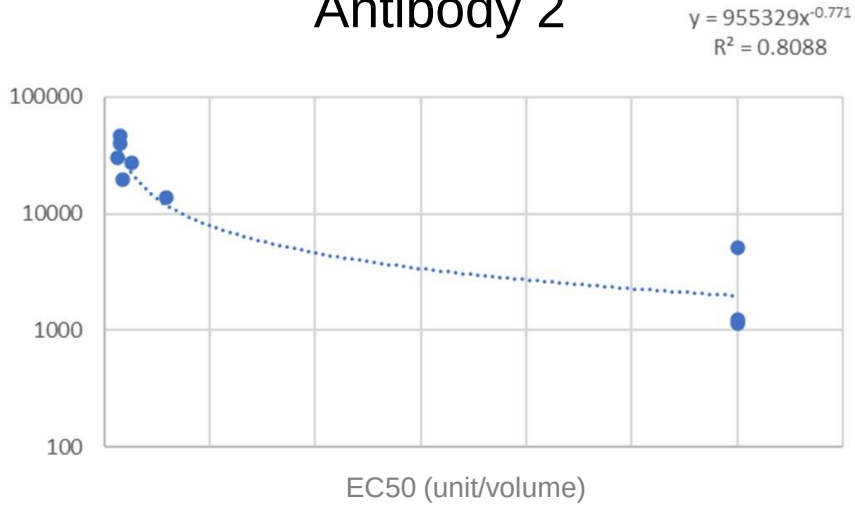


Antibody 2 Shows Good Correlation Between Neutralization Titers and Expression

Antibody 1



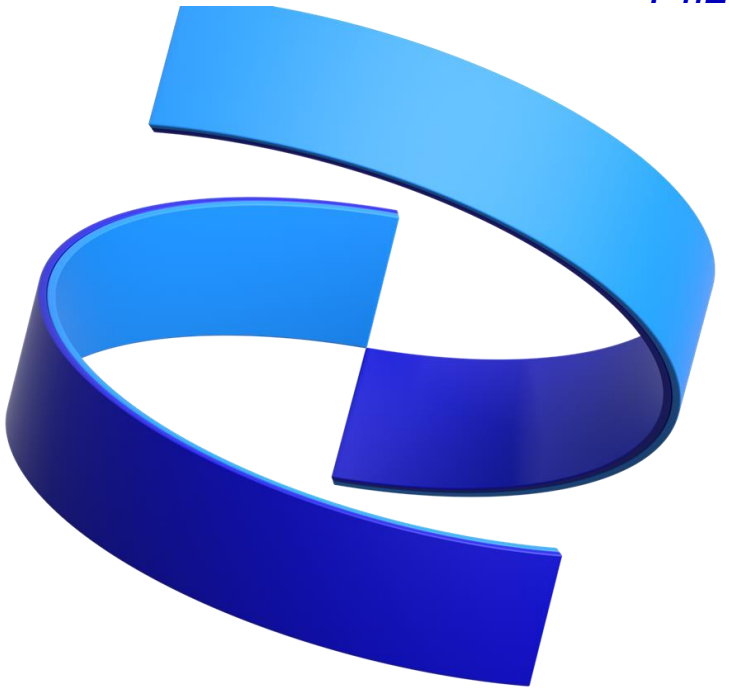
Antibody 2



Summary

- **Introduction to modern potency assay techniques and new vaccine technology in an established space**
  - Establishing the *In-vitro* Expression (IVE) Assay for potency expression of mRNA vaccine products
  - Powerful, fast, and reliable tool to confirm protein presence and in-vitro expression by flow cytometry
- **Approaches and challenges to "platform" release methods for quick validation of new strains**
  - Method development entails reagent selection/generation and assay conditions optimization
  - Screen of mRNA vaccine variants allows to address strain updates and form multivalent strategies
- **Lessons for Rapid Response in a Pandemic Setting and IVE Assay Applications**
  - Target info, experimental design, cell type choice are critical aspects to consider
  - Screen mRNA vaccine product formulation
  - Track temperature stability of potency expression over time
  - Compare expression of benchmark vs mutant constructs
- **Selection of assay format (*in-vitro* vs. *in-vivo*) & Correlation of *in-vitro* and *in-vivo* data (antibody response)**
  - Neutralization response from animals and IVE data - strong correlation with high titers
  - Each antigen is unique but you have to do the initial studies to establish connection
  - Monitoring the right epitope with right antibody is critical

Acknowledgements



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Thank you

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