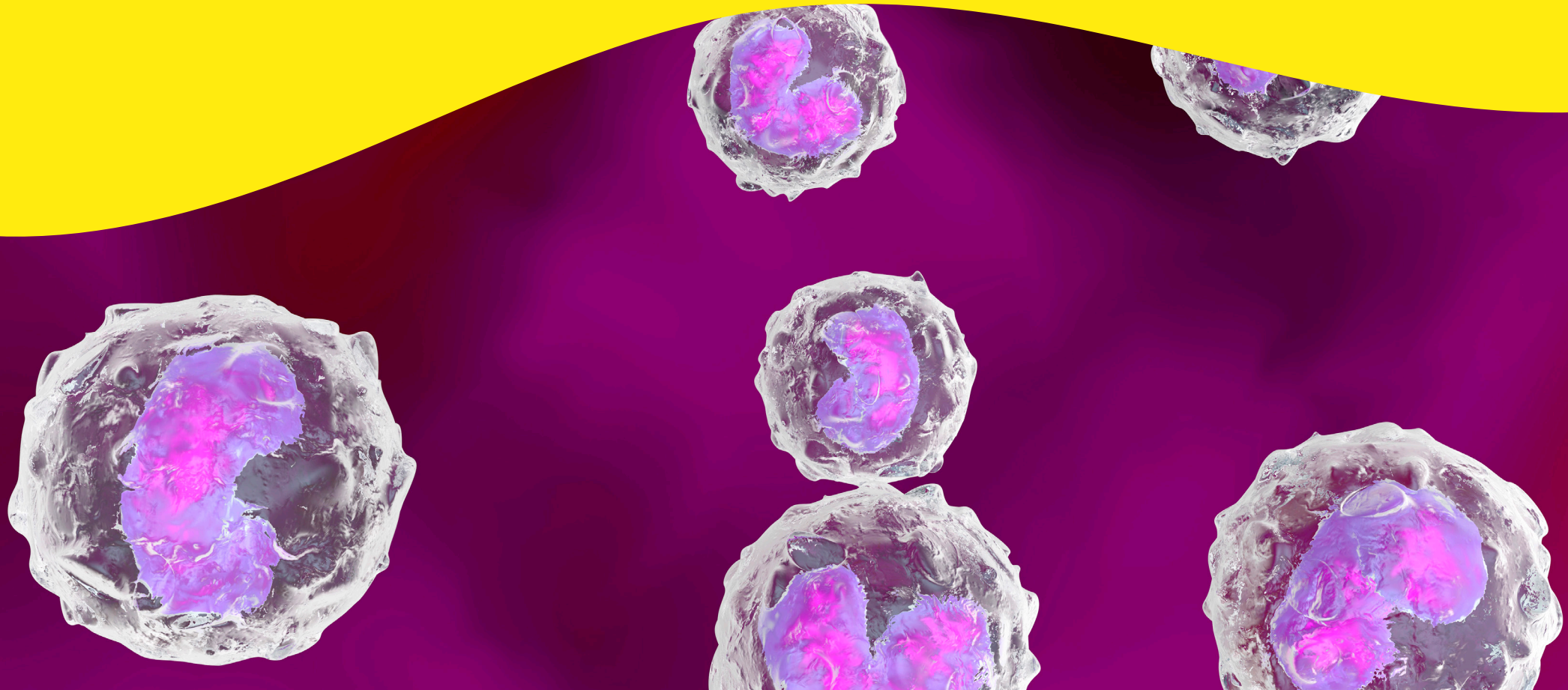


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AlphaLISA Cytokine Assays

Guide to Success with AlphaLISA™



CYTOKINE DETECTION WITH ALPHALISA

AlphaLISA Features and Benefits

What is AlphaLISA?

AlphaLISA™ is a bead-based assay technology used to study biomolecular interactions in a microplate format. The acronym "Alpha" stands for amplified luminescent proximity homogeneous assay.

How does AlphaLISA work?

The binding of molecules captured on the beads leads to an energy transfer from one bead to the other, ultimately producing a luminescent/

fluorescent signal.

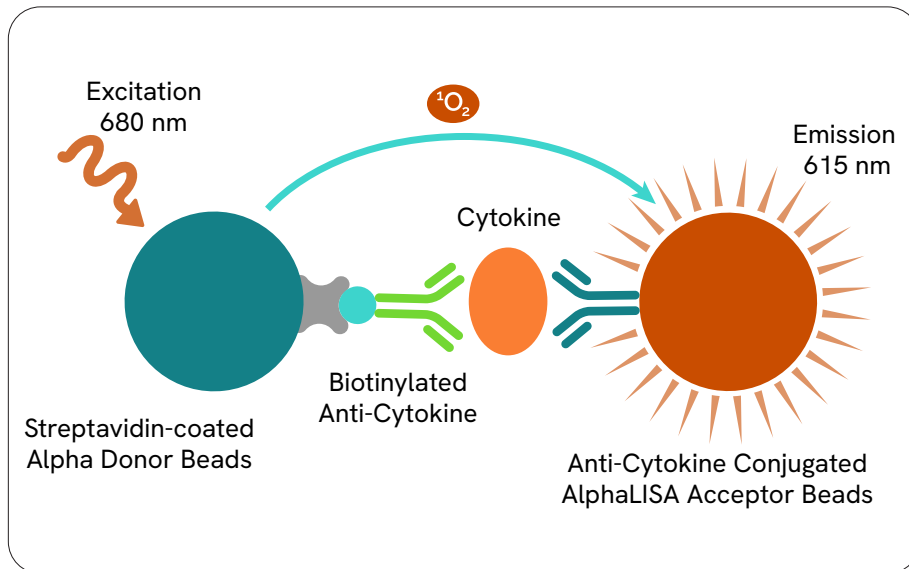
AlphaLISA for Cytokine Detection

Alpha technology is optimal for detecting proteins in cell culture, supernatant, cell lysate, serum, plasma, urine, saliva, and CSF - making it the ideal homogenous immunoassay for cytokine research.

A large selection of cytokine kits are available, including interleukins (IL), interferons (IFN), chemokines, lymphokines, and TNF, with many options for kits specific to human, non-human primate, mouse, rat, porcine, bovine, and ovine cytokines.

Features and Benefits:

- High sensitivity to detect down to femtogram levels of targets utilizing just 5 µL of sample
- Large dynamic range up to 4-5 logs, requiring minimal sample dilution
- Homogeneous assay format without any washing steps
- Fast results within 3 hours
- Ability to measure a variety of complex sample types
- Automation compatible



AlphaLISA Assay Principle

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- Choosing the Right Plate Reader

GETTING TO KNOW YOUR ALPHALISA CYTOKINE KIT

Kit Contents and Workflow

What's in your kit?

- AlphaLISA Anti-analyte Acceptor Beads
- Streptavidin (SA)-coated Donor Beads
- Biotinylated Anti-analyte Antibody
- Lyophilized Analyte
- AlphaLISA Assay Buffer

Tips:

- Manuals and lot specific data sheets can be found at www.revvy.com
- Optimized protocols are provided for each kit, varying in time to run an assay and sensitivity

Workflow for Cytokine Detection

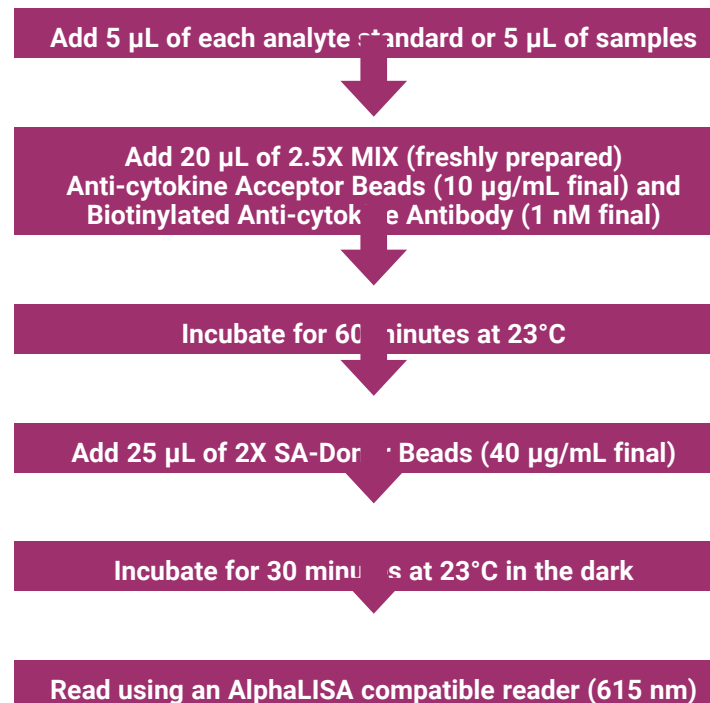


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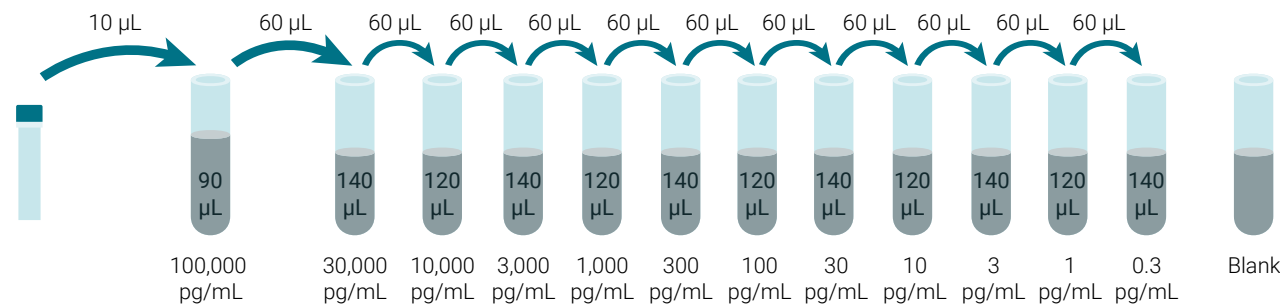


GETTING TO KNOW YOUR ALPHALISA CYTOKINE KIT

Preparing the Standard Curve

Each AlphaLISA Cytokine Detection Kit contains a lyophilized recombinant protein standard that is used to prepare a standard curve.

It is recommended to dilute the standard in a 12-point half-log dilution series in AlphaLISA assay buffer, which allows coverage of the extended dynamic range of AlphaLISA assays (5.5 logs) in just 12 dilutions.



← 5.5 logs [cytokine standard] →

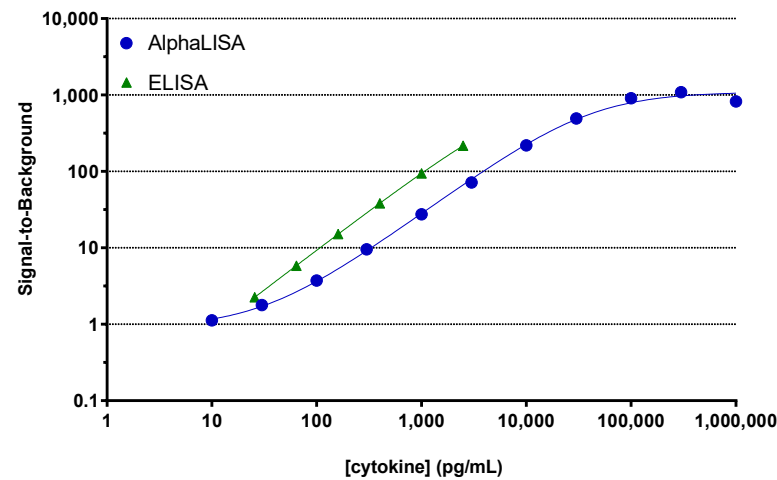


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GETTING TO KNOW YOUR ALPHALISA CYTOKINE KIT

Graphing the Standard Curve

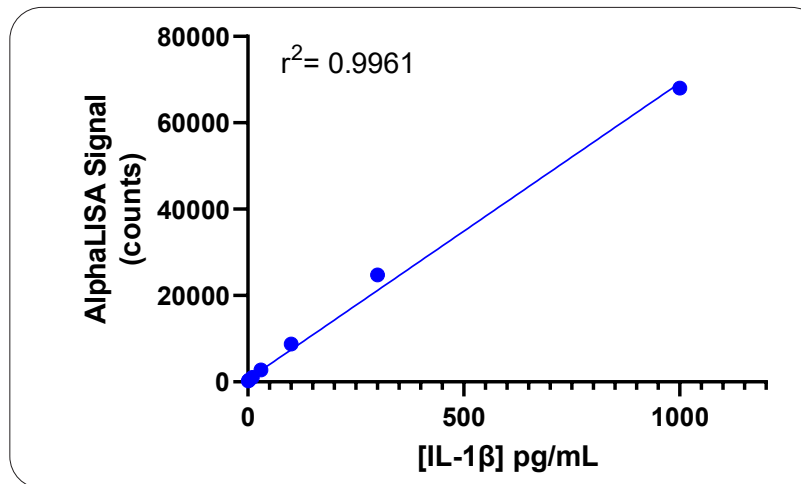
AlphaLISA standard curve data can be fit to either a linear curve (using just the linear portion of your data) or a dose-response curve. A dose response curve (or sigmoidal or 4PL curve), using $1/Y^2$ weighting is typically used to process AlphaLISA immunoassay data in order to take advantage of the full dynamic range of the assay.

Once graphed, the standard curve can be used to calculate the lower detection limit (LDL) and lower limit of quantification (LLOQ) for the assay.

The LDL corresponds to the lowest amount of analyte in a sample that can be discriminated from the background. This value for AlphaLISA cytokine assays can be determined by adding three standard deviations to the mean AlphaLISA signal of twelve zero replicates and calculating the corresponding concentration.

The LLOQ is the lowest amount of analyte in a sample which can be quantitatively determined with precision and accuracy. The LLOQ of AlphaLISA cytokine assays can be determined by adding ten standard deviations to the mean AlphaLISA signal of twelve zero replicates and calculating the corresponding concentration.

Linear Plot



Log: Log Plot- allows for a wider dynamic range

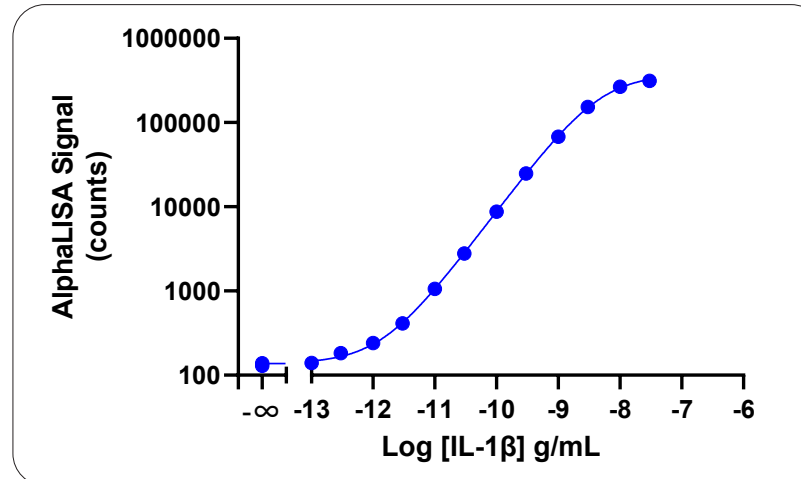


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GETTING TO KNOW YOUR ALPHALISA CYTOKINE KIT

Interpolating Concentrations From Unknown Samples

To quantify the cytokine concentration in unknown samples, the signal generated from the sample is compared to the standard curve. It is important to prepare your standard curve (dilute your standard) in the same or similar matrix as your sample. In the example below, the standard curve was prepared in the same culture media (RPMI + 10% FBS), the same matrix the cell supernatant samples were prepared in.

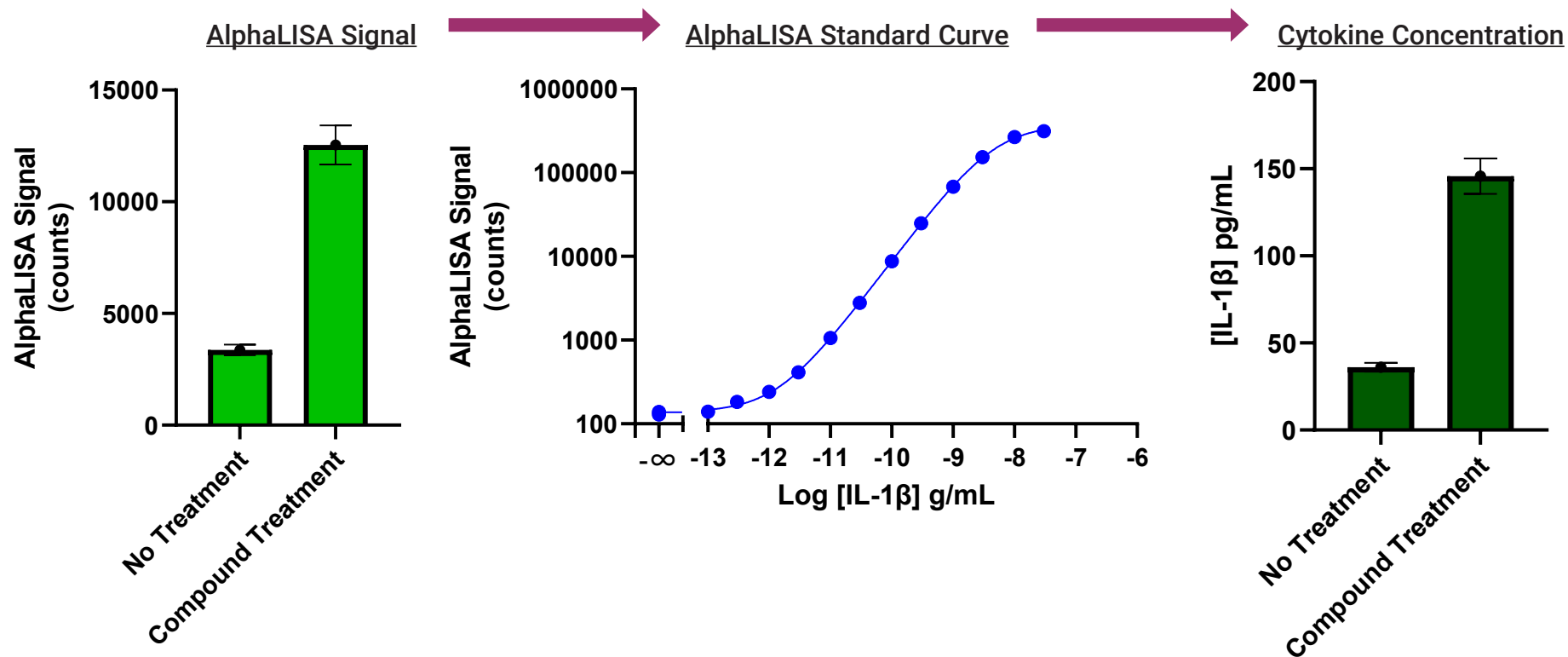


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GETTING TO KNOW YOUR ALPHALISA CYTOKINE KIT

Be Mindful of the Hook Effect

Like any sandwich immunoassay, assay signal increases proportionally to the analyte being quantified until a plateau is reached.

In an Alpha assay, if the amount of cytokine is significantly higher than the amount of detection reagents present in the well, the signal goes down (illustrated in the figure below). This situation is known as the hook effect.

The experimental conditions (e.g. cell density, sample dilution) must be optimized for each cytokine kit and experimental model to avoid the hook effect and assure that cytokine concentrations measured are within the detection range of each assay kit.

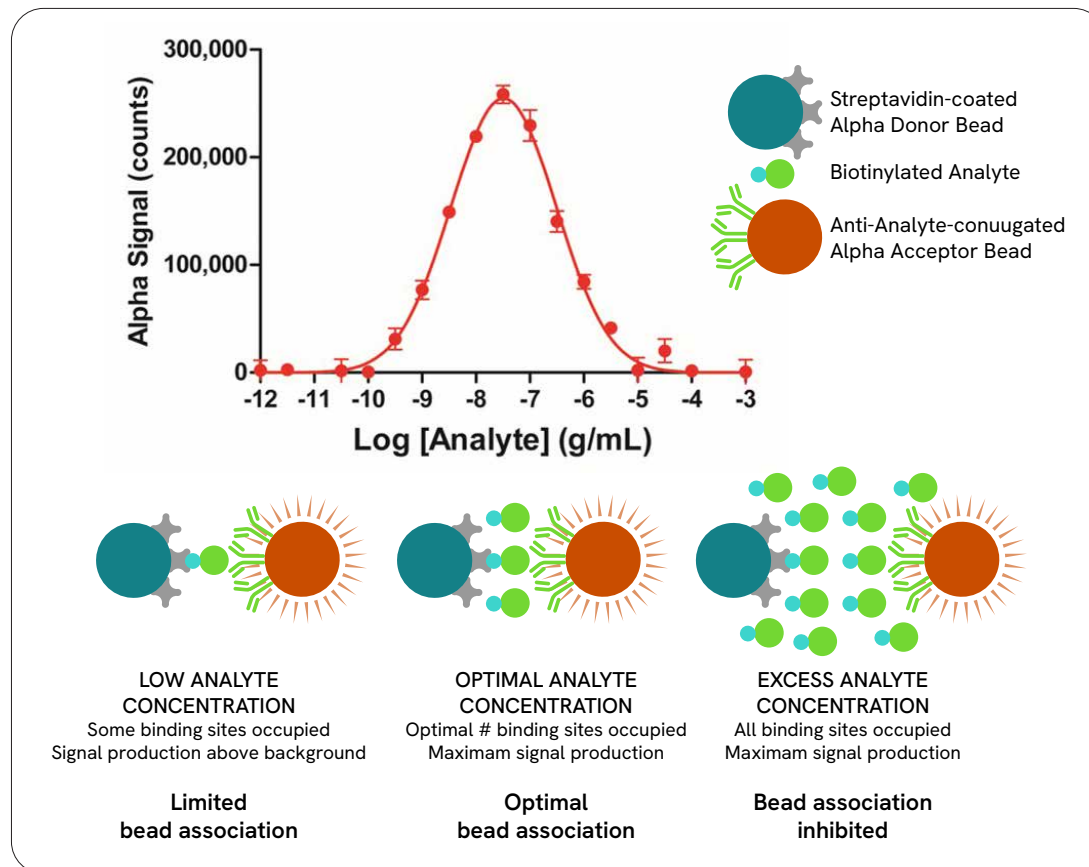


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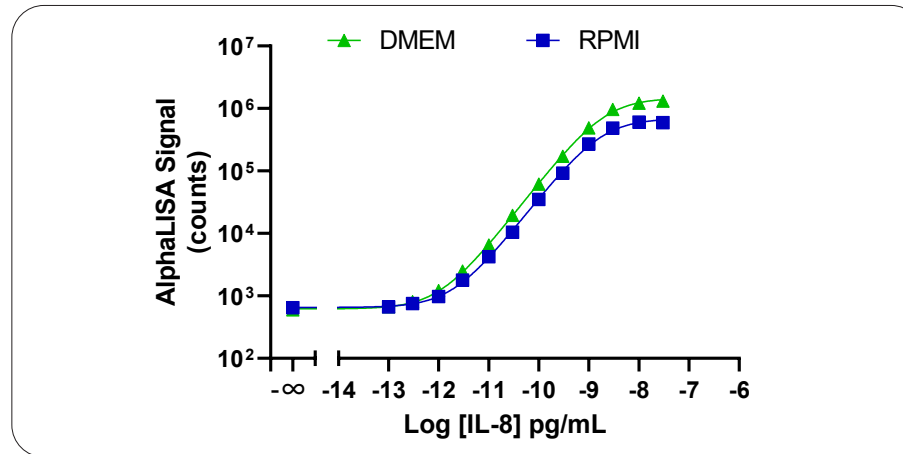
WORKING WITH CELL SUPERNATANT SAMPLES

AlphaLISA Cytokine Assays in Different Cell Culture Media

When using AlphaLISA to detect cytokines in cell supernatant samples, depending on the composition of the cell culture medium, the limit of quantification may vary.

It is recommended that the standard analytes are diluted in the same cell culture medium used for sample preparation to ensure satisfactory results and more accurate quantitation.

On the right is an example of an AlphaLISA cytokine standard curve prepared in two different cell culture media: DMEM and RPMI. Assay parameters for each matrix are displayed in the table.



	DMEM	RPMI
LDL	0.17 pg/mL	0.28 pg/mL
LLOQ	0.62 pg/mL	0.96 pg/mL
Maximum Signal	1,298,560	593,908
Minimum Signal	596	647

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WORKING WITH CELL SUPERNATANT SAMPLES

Cell Density Optimization

For cell-based assays, cell density is a key parameter that must be optimized to suit the dynamic range of each cytokine kit while also respecting the intrinsic biology of the cellular model being studied. We recommend running an experiment to test different cell seeding densities of your cell line to ensure the amount of cytokine being secreted is detectable in the AlphaLISA assay.

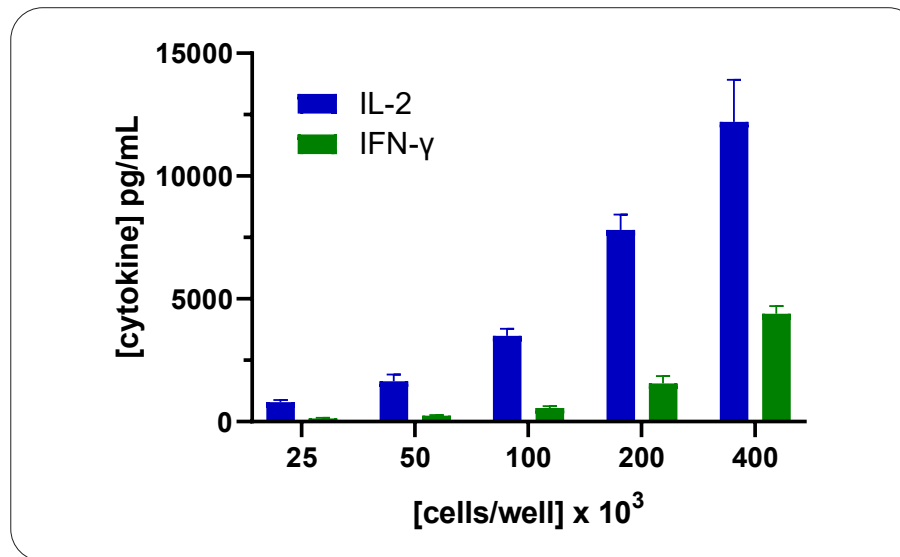
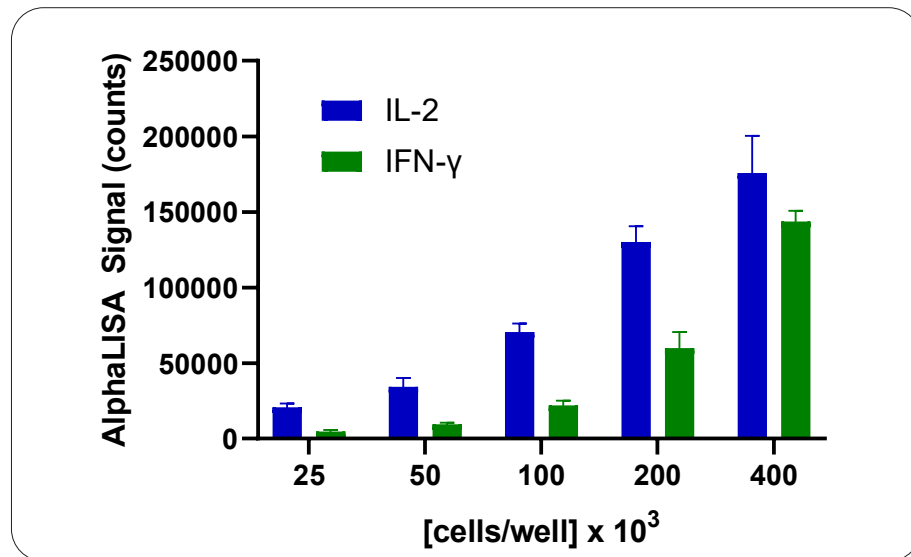


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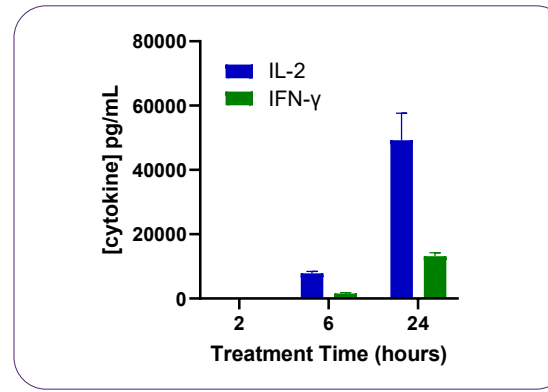
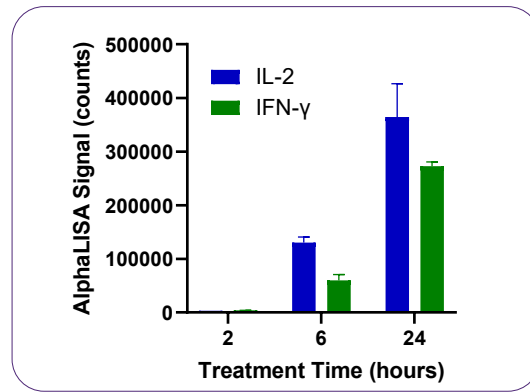
WORKING WITH CELL SUPERNATANT SAMPLES

Cell Treatment Optimization

Additional parameters that may need to be optimized are the duration of compound treatment and the concentration of compound being used.

TIME-COURSE OF COMPOUND TREATMENT

Determine the optimal duration of treatment either with agonists or antagonists.



OPTIMIZATION OF COMPOUND CONCENTRATION

Determination of pharmacological parameters such as IC₁₀ or IC₈₀ may be important to identify new agonists or antagonists.

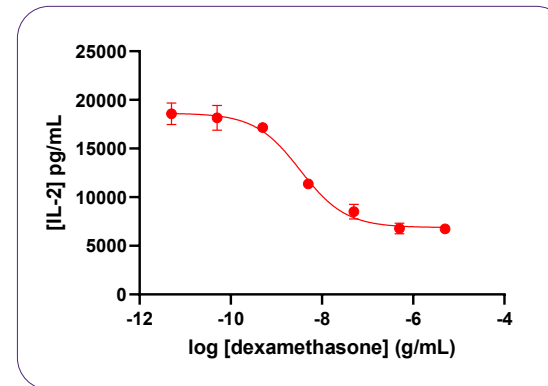
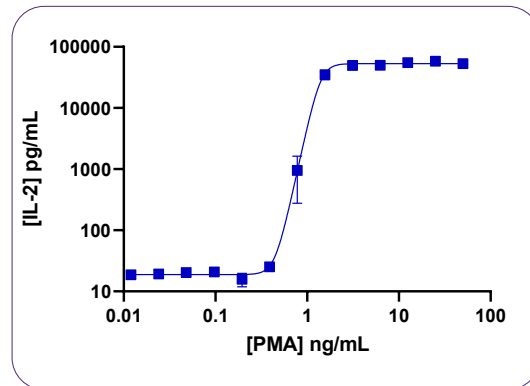


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WORKING WITH MORE COMPLEX SAMPLE TYPES

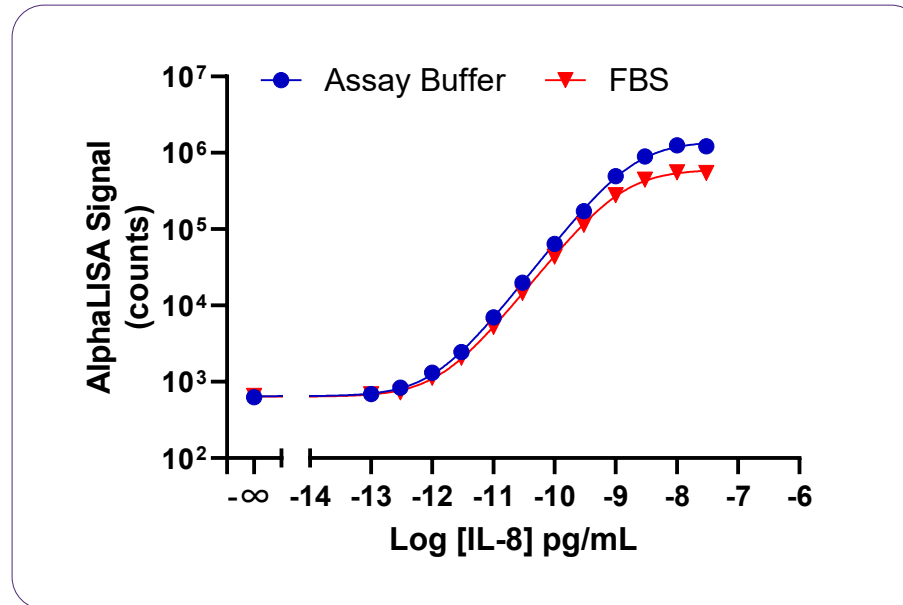
Matrix Compatibility Testing

AlphaLISA Cytokine Kits are often used for quantitative detection in complex sample types including serum and plasma, as well as other biological fluids including cerebrospinal fluid (CSF), bronchoalveolar lavage fluid (BALF), urine, and saliva. Each of these fluids has a unique protein composition and will require specific validation.

For quantitation of cytokines in complex samples, it is necessary to create a standard curve of your analyte in a matrix that closely matches your samples.

For example, if you are working with serum samples, you will need to create a standard curve of your analyte in analyte-depleted serum or something similar, such as fetal bovine serum (FBS).

On the right is an example of an AlphaLISA cytokine standard curve prepared in assay buffer and FBS, with assay parameters for each matrix displayed in the table.



	Assay Buffer	FBS
LDL	0.06 pg/mL	0.22 pg/mL
LLOQ	0.27 pg/mL	0.64 pg/mL
Maximum Signal	1,217,919	545,509
Minimum Signal	629	653

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ADDITIONAL CONSIDERATIONS

Choosing the Right Cytokine Kit Format

Many AlphaLISA Cytokine Kits are available in two different detection formats depending on the need for compatibility with samples containing very high levels of endogenous biotin.

AlphaLISA Biotin-Free Cytokine Kits utilize a DIG-antibody and anti-DIG acceptor beads, making these assays impervious to biotin interference.

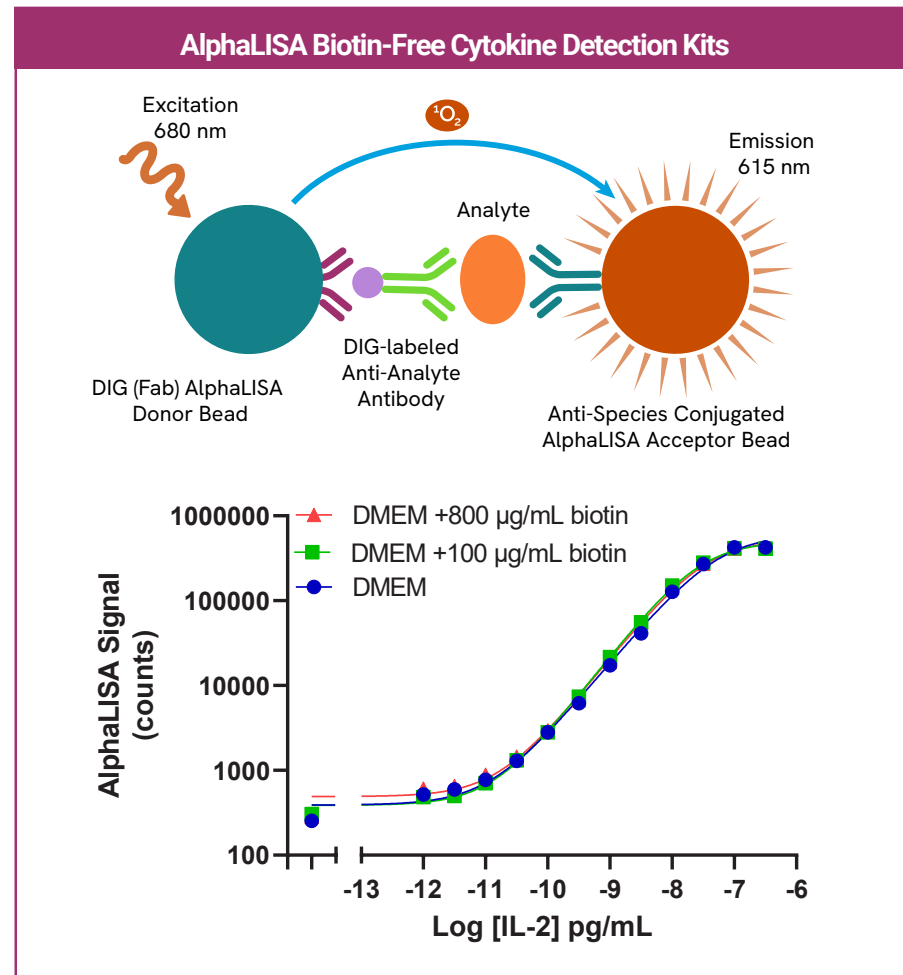
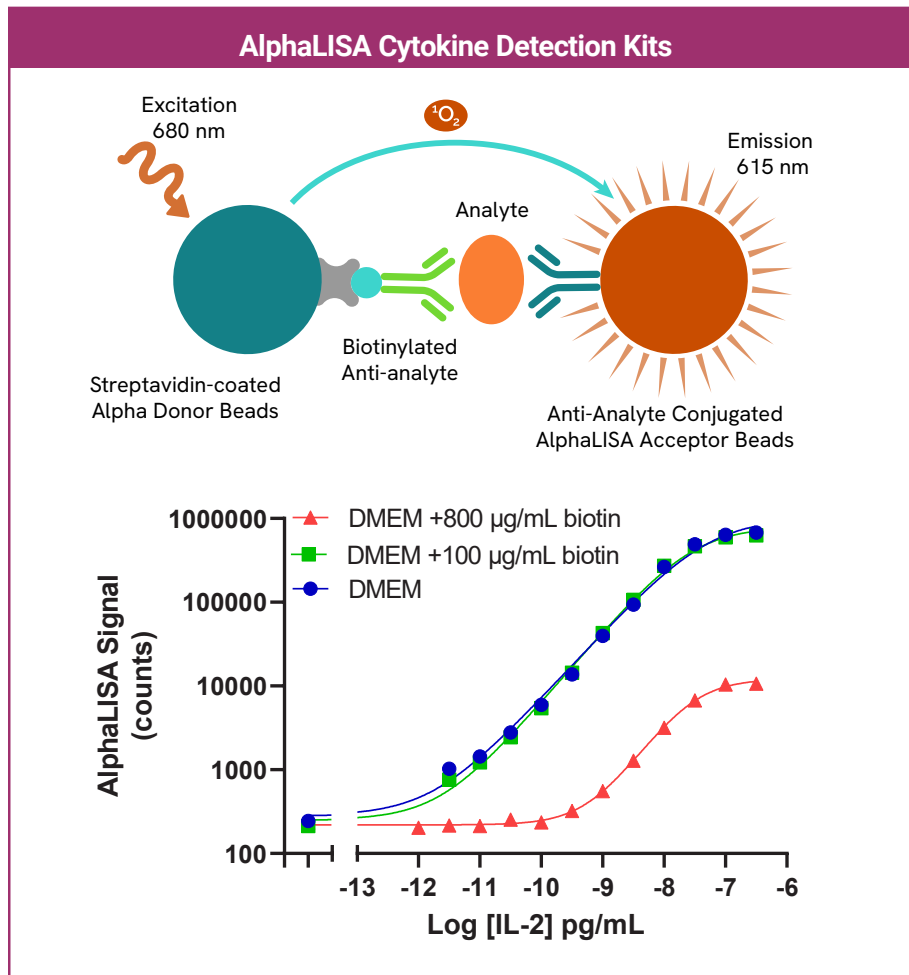


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ADDITIONAL CONSIDERATIONS

Choosing the Right Microplate

AlphaLISA Cytokine Kits provide the flexibility to scale-up or scale-down total assay volume as needed with compatibility with 96-well, 384-well, and 1536-well microplates.

AlphaLISA cytokine assays are tested on both white OptiPlates™ and light gray AlphaPlates™.

Light gray AlphaPlates plate reduce the potential for well-to-well crosstalk and result in more consistent AlphaLISA data, particularly when a large portion of the wells in the microplate are being utilized.

The table below provides a list of recommended Revvity microplates that are compatible with AlphaLISA cytokine assays.

	Recommended Microplate	Catalog Number*	Recommended AlphaLISA Assay Volume
White Plates	OptiPlate-96	6005290	100 µL
	OptiPlate-384	6007290	25-50 µL
	½ AreaPlate-96	6002290	50-100 µL
	ProxiPlate-384 Plus	6008280	10-20 µL
Light Gray Plates	½ Area AlphaPlate-96	6002350	50-100 µL
	AlphaPlate-384	6005350	25-50 µL
	AlphaPlate-384 Shallow Well	6008350	10-20 µL
	AlphaPlate-1536	6004350	5-10 µL

*multiple size cases are available



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ADDITIONAL CONSIDERATIONS

Choosing the Right Plate Reader

AlphaLISA technology requires excitation at 680 nm and reading emission at 615 nm. A laser excitation source, rather than a Xenon Flashlamp, is needed, as an intense light source is required to generate signal. Many basic fluorescence plate readers are not equipped to run Alpha assays, you need the Alpha option installed.

Revvity offers 3 different multimode readers, all are compatible with AlphaLISA technology. AlphaLISA assays are also compatible with multimode readers from other providers as well.



EnVision™

Exceptional speed and sensitivity simultaneously, ideal for labs needing high throughput combined with robust performance



EnSight™

Fast well-imaging and multimode detection, all in one instrument, to gain deeper insights



VICTOR™ Nivo™

Industry's smallest multimode plate reader for everyday biochemical and cell-based assays

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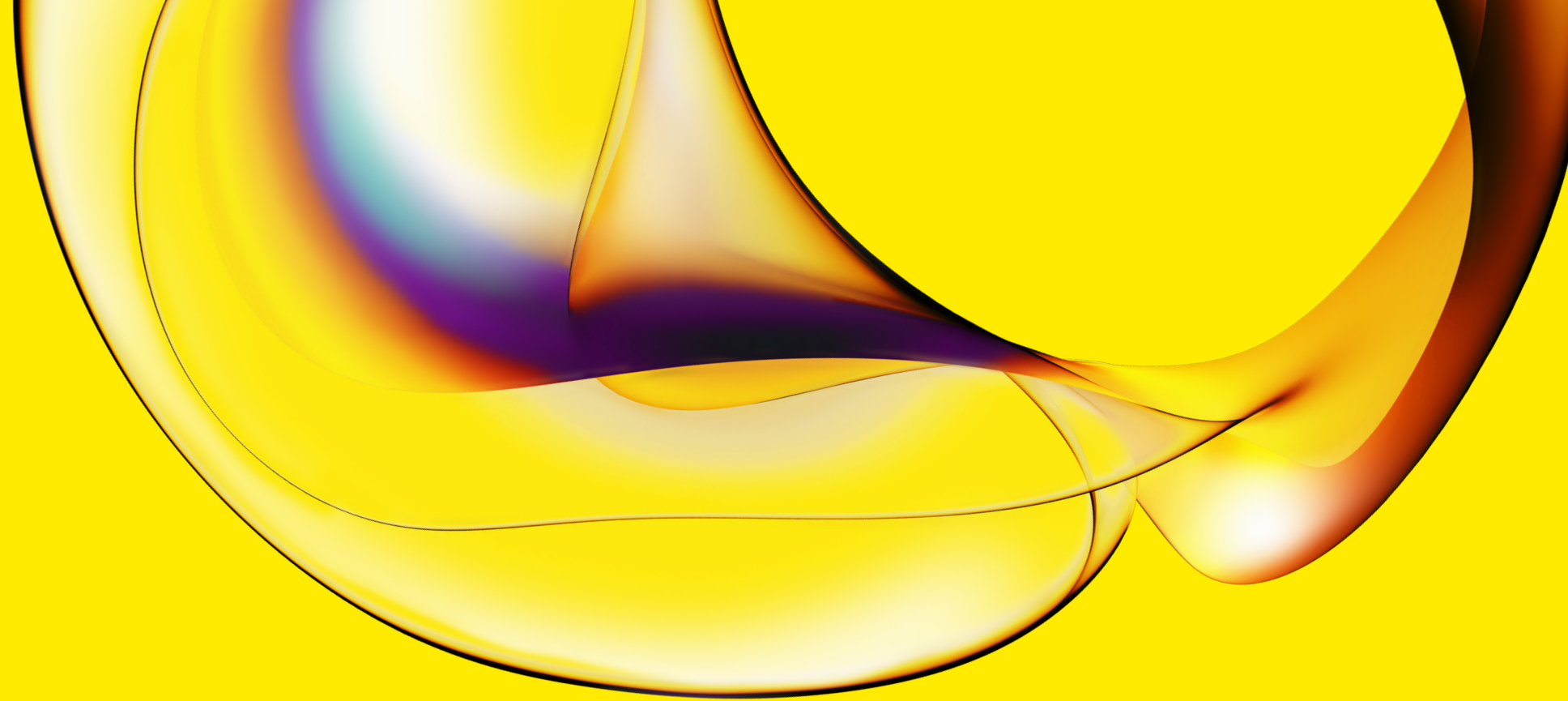
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