MANUAL

AlphaLISA® High Performance (HP) Mouse Interleukin 1 beta (IL1β) Detection Kit

Product number: AL592HV/C/F

Research Use Only. Not for use in diagnostic procedures.

Product Information

Application: This kit is designed for the quantitative determination of mouse IL1β using a homogeneous no wash AlphaLISA assay. This assay is also compatible with rat IL1β but requires the use of a recombinant rat standard (not provided in the kit) to generate a standard curve.

Kit contents: The kit contains 5 components: AlphaLISA Acceptor beads coated with mouse Interleukin 1 beta Antibody, Streptavidin-coated Donor beads, Biotinylated mouse Interleukin 1 beta antibody, Lyophilized mouse Interleukin 1 beta analyte standard and 10X AlphaLISA Immunoassay Buffer.

Sensitivity: Lower Detection Limit (LDL): 0.79 pg/mL;
Lower Limit of Quantification (LLOQ): 2.60 pg/mL
EC_{50}: 9.70 ng/mL

Dynamic Range: 0.79– 30 000 pg/mL

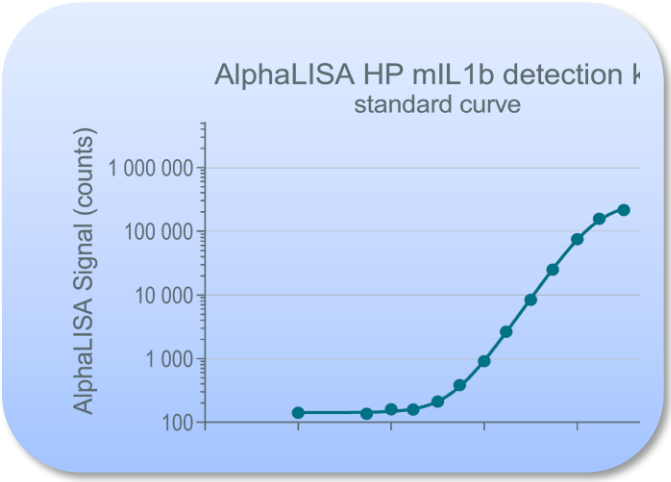


Figure 1. Typical sensitivity curve in AlphaLISA Immunoassay Buffer. The data was generated using a gray AlphaPlate™-384 microplate and the EnVision® Multilabel Plate Reader 2102 with Alpha option.

Storage: Store kit in the dark at 4 ºC. For reconstituted analyte, aliquot and store at -20 ºC. Avoid freeze-thaw cycles.

Stability: This kit is stable for at least 24 months from the date of manufacture when stored in its original packaging and the recommended storage conditions.

Analyte of Interest

The mouse Interleukin 1 beta (mIL1ß) is produced as a 269 amino acid precursor that matures by proteolysis to its 152 amino acid active form. In humans, IL1α and IL1ß are central players of the immune response, displaying roles in inflammation both at local and systemic levels. IL1ß is functionally equivalent to IL1α. Its production has been reported in many cell types including brain cells, as well as monocytic and peripheral blood mononuclear cells. Among the biological activities of IL1 is the stimulation of T-helper cells, which then secrete IL2 and express IL2 receptor. IL1 acts directly on B-cells, promoting their proliferation and the synthesis of immunoglobulins. It supports tumor cytotoxicity mediated by monocytes and induces tumor regression. It has been shown that IL1 also promotes wound healing.

Description of the AlphaLISA Assay

AlphaLISA technology allows the detection of molecules of interest in buffer, cell culture media, serum and plasma in a highly sensitive, quantitative, reproducible and user-friendly mode. In this AlphaLISA assay, a biotinylated anti-mouse interleukin 1 beta antibody binds to the streptavidin coated AlphaLISA Donor beads, while the anti-mouse interleukin 1 beta antibody is conjugated to AlphaLISA Acceptor beads. In the presence of mouse or rat interleukin 1 beta (IL1ß), the beads come into proximity. The excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer within the Acceptor beads, resulting in emission with λ_{max} at 615 nm (Figure 2).

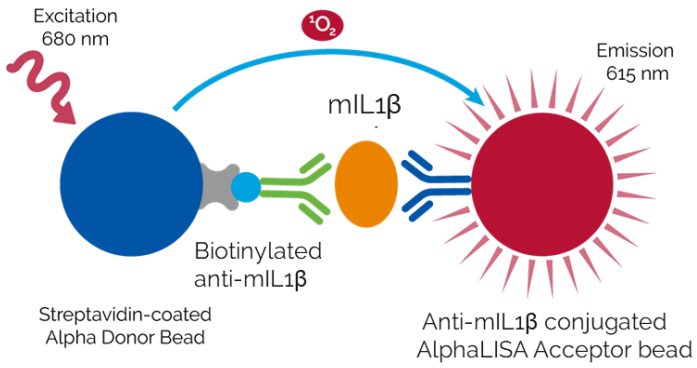


Figure 2. AlphaLISA HP mouse interleukin-1beta (mIL1ß) Detection Assay Principle.

Precautions

- The Alpha Donor beads are light-sensitive. All the other assay reagents can be used under normal light conditions. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco) can be applied to light fixtures.
- Take precautionary measures to avoid contamination of the reagent solutions.
- The biotinylated Anti-mouse IL1ß antibody contains sodium azide. Contact with skin or inhalation should be avoided.

Kit Content: Reagents and Materials

Kit components	AL592HV 100 assay points***	AL592C 500 assay points***	AL592F 5000 assay points***
AlphaLISA Anti-mouse IL1ß Acceptor beads stored in PBS, 0.05% Kathon CG/ICP, pH 7.2	25 µL @ 5 mg/mL (1 brown tube, white cap)	50 µL @ 5 mg/mL (1 brown tube, white cap)	500 µL @ 5 mg/mL (1 brown tube, white cap)
Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Kathon CG/ICP, pH 7.4	100 µL @ 5 mg/mL (1 brown tube, black cap)	200 µL @ 5 mg/mL (1 brown tube, black cap)	2 x1 mL @ 5 mg/mL (1 brown tube, black cap)
Biotinylated Anti-mouse IL1ß Antibody stored in PBS, 0.1% Tween-20, 0.05% NaN_3, pH 7.4	25 µL @ 500 nM (1 tube, black cap)	50 µL @ 500 nM (1 tube, black cap)	500 µL @ 500 nM (1 tube, black cap)
Lyophilized mouse IL1ß Analyte*	0.1 µg (1 tube, clear cap)	0.1 µg (1 tube, clear cap)	0.1 µg (1 tube, clear cap)
AlphaLISA Immunoassay Buffer (10X) **	2 mL, 1 small bottle	10 mL, 1 small bottle	100 mL, 1 large bottle

* Reconstitute lyophilized analyte in 100 µL Milli-Q® grade H_2O. The reconstituted analyte should be used within 60 minutes or aliquoted into screw-capped 0.5 mL polypropylene vials and stored at -20°C for future experiments. The aliquoted analyte at -20°C is stable up to 28 days. Avoid freeze-thaw cycles. One vial contains an amount of analyte sufficient for performing 10 standard curves. Additional vials can be ordered separately (cat # AL592S).

** Extra buffer can be ordered separately (cat # AL000C: 10 mL, cat # AL000F: 100 mL).

*** The number of assay points is based on an assay volume of 100 µL in 96-well plates or 50 µL in 384-well assay plates using the kit components at the recommended concentrations.

Sodium azide should **not** be added to the stock reagents. High concentrations of sodium azide (> 0.001% final in the assay) might decrease the AlphaLISA signal. Note that sodium azide from the biotinylated Anti-mouse IL1ß antibody stock solution will not interfere with the AlphaLISA signal (0.0001% final in the assay).

Specific additional required reagents and materials:

The following materials are recommended:

Item	Suggested source	Catalog #
TopSeal™-A Plus Adhesive Sealing Film	Revvity Inc.	6050185
EnVision®-Alpha Reader	Revvity Inc.	-

Recommendations

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BELOW BEFORE USE

- The volume indicated on each tube is guaranteed for single pipetting. Multiple pipetting of the reagents may reduce the theoretical amount left in the tube. To minimize loss when pipetting beads, it is preferable not to pre-wet the tip.
- Centrifuge all tubes (including lyophilized analyte) before use to improve recovery of content (2000*g*, 10-15 sec). Re-suspend all reagents by vortexing before use.
- Use Milli-Q® grade H_2O to dilute 10X AlphaLISA Immunoassay Buffer and to reconstitute the lyophilized analyte.
- When diluting the standard or samples, change tips between each standard or sample dilution. When loading reagents in the assay microplate, change tips between each standard or sample addition and after each set of reagents.
- When reagents are added to the microplate, make sure the liquids are at the bottom of the well.
- Small volumes may be prone to evaporation. It is recommended to cover microplates with TopSeal-A Adhesive Sealing Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Film in place.
- The AlphaLISA signal is detected with an EnVision Multilabel Plate Reader equipped with the Alpha option using the AlphaScreen standard settings (e.g. Total Measurement Time: 550 ms, Laser 680 nm Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).
- AlphaLISA signal will vary with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for each plate.
- The standard curves shown in this technical data sheet are provided for information only. A standard curve must be generated for each experiment.

Assay Procedure

- The protocol described below is an example for generating one standard curve in a 50 µL final assay volume (48 wells, triplicate determinations). The protocols also include testing samples in 452 wells. If different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly, as shown in the table below. These calculations do not include excess reagent to account for losses during transfer of solutions or dead volumes.
- The standard dilution protocol is provided for information only. As needed, the number of replicates or the range of concentrations covered can be modified.
- Use of four background points in triplicate (12 wells) is recommended when LDL/LLOQ is calculated. One background point in triplicate (3 wells) can be used when LDL/LLOQ is not calculated.

		Volume				
Format	**# of data points**	**Final**	**Sample**	**MIX AlphaLISA AccBeads + biotinylated Ab**	**SA-Donor beads**	**Plate recommendation**
AL592HV	100	100 µL	10 µL	40 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
AL592C	250	100 µL	10 µL	40 µL	50 µL	White OptiPlate-96 (cat # 6005290)
	500	50 µL	5 µL	20 µL	25 µL	½ Area AlphaPlate-96 (cat # 6002350) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate™-384 (cat # 6005350)
	1 250	20 µL	2 µL	8 µL	10 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate™-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	2 500	10 µL	1 µL	4 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)
AL592F	5 000	50 µL	5 µL	20 µL	25 µL	½ Area AlphaPlate-96 (cat # 6002350) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate-384 (cat # 6005350)
	12 500	20 µL	2 µL	8 µL	10 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	25 000	10 µL	1 µL	4 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)

The 2-Step Standard Protocol described below is for 500 assay points including one standard curve (48 wells) and samples (452 wells). *If different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly.*

1) Preparation of 1X AlphaLISA Immunoassay Buffer:
   Add 5 mL of 10X AlphaLISA Immunoassay Buffer to 45 mL Milli-Q® grade H_2O.

2) Preparation of mouse IL1ß analyte standard dilutions:
   a. Reconstitute lyophilized mouse IL1ß (0.1 µg) analyte in 100 µL Milli-Q® grade H_2O. The remaining reconstituted analyte should be aliquoted immediately and stored at -20 ºC for future assays (see page 3 for more details).
   b. A standard curve must be generated for each experiment. The standard curve should be performed in a similar matrix diluent as the samples (e.g. cell culture media for cell supernatant samples, FBS for serum samples). Use of the 1X AlphaLISA Immunoassay Buffer is recommended as a diluent to confirm assay performance.
   c. Prepare standard dilutions as follows in 1X AlphaLISA Immunoassay Buffer (change tip between each standard dilution):

Tube	Vol. of mouse IL1b (µL)	Vol. of diluent (µL) *	[mouse IL1b] in standard curve	
			(g/mL in 5 µL)	(pg/mL in 5 µL)
A	10 µL of reconstituted mouse IL1b	90	1.00E-07	100 000
B	60 µL of tube A	140	3.00E-08	30 000
C	60 µL of tube B	120	1.00E-08	10 000
D	60 µL of tube C	140	3.00E-09	3 000
E	60 µL of tube D	120	1.00E-09	1 000
F	60 µL of tube E	140	3.00E-10	300
G	60 µL of tube F	120	1.00E-10	100
H	60 µL of tube G	140	3.00E-11	30
I	60 µL of tube H	120	1.00E-11	10
J	60 µL of tube I	140	3.00E-12	3
K	60 µL of tube J	120	1.00E-12	1.0
L	60 µL of tube K	140	3.00E-13	0.3
M ** (background)	0	100	0	0
N ** (background)	0	100	0	0
O ** (background)	0	100	0	0
P ** (background)	0	100	0	0

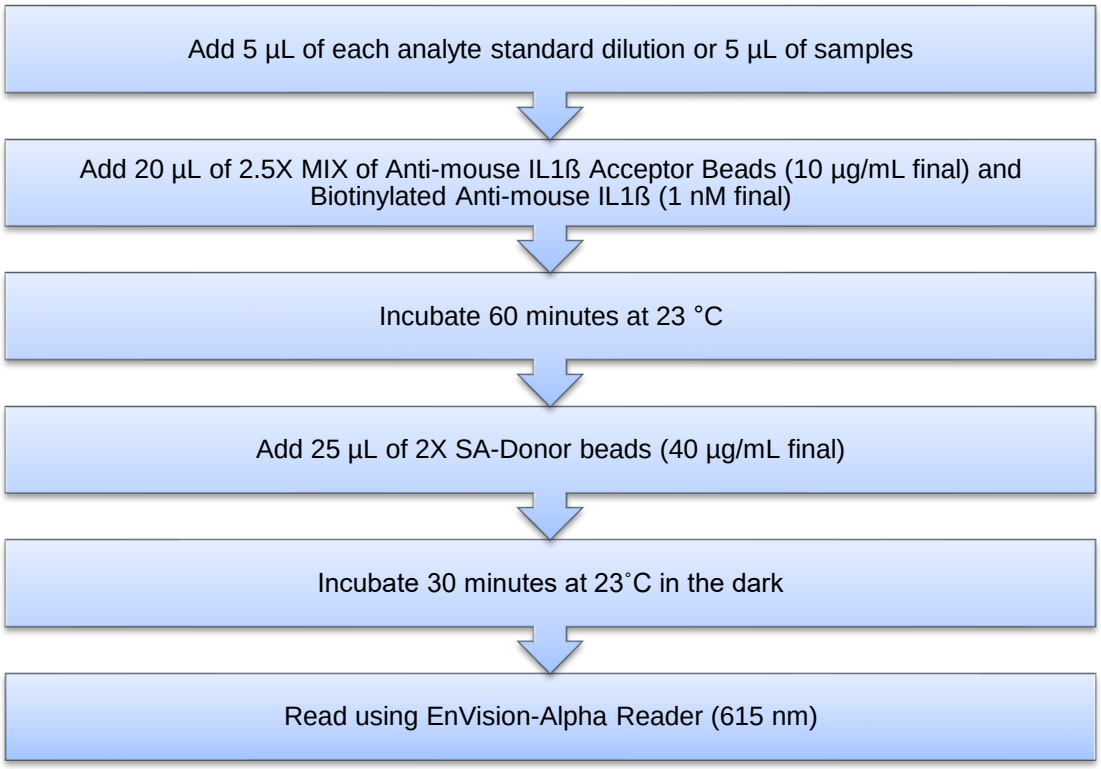
* Dilute standards in diluent (e.g. 1X AlphaLISA Immunoassay Buffer).
At low concentrations of analyte, a significant amount of analyte can bind to the vial. Therefore, load the analyte standard dilutions in the assay microplate within 60 minutes of preparation.

** Four background points in triplicate (12 wells) are used when LDL is calculated. If LDL does not need to be calculated, one background point in triplicate can be used (3 wells).

3) Preparation of 2.5X MIX AlphaLISA Anti-mouse IL1ß Acceptor beads (25 µg/mL) + Biotinylated Anti- mouse IL1ß Antibody (2.5 nM):
   a. Prepare just before use.
   b. Add 50 µL of 5 mg/mL AlphaLISA Anti-mouse IL1ß Acceptor Bead and 50 µL of 500 nM Biotinylated Anti mouse IL1ß Antibody to 9 900 µL of 1X AlphaLISA Immunoassay Buffer.

4) Preparation of 2X Streptavidin (SA) Donor beads (80 µg/mL):
   a. Prepare just before use.
   b. Keep the beads under subdued laboratory lighting.
   c. Add 200 µL of 5 mg/mL SA-Donor beads to 12 300 µL of 1X AlphaLISA Immunoassay Buffer.

5) In a gray AlphaPlate (384 wells):



Data Analysis

- Calculate the average count value for the background wells.
- Generate a standard curve by plotting the AlphaLISA counts versus the concentration of analyte. A log scale can be used for either or both axes. No additional data transformation is required.
- Analyze data according to a nonlinear regression using the 4-parameter logistic equation (sigmoidal dose-response curve with variable slope) and a $1/Y^2$ data weighting (the values at maximal concentrations of analyte after the hook point should be removed for correct analysis).
- The LDL is calculated by interpolating the average background counts (12 wells without analyte) + 3 x standard deviation value (average background counts + (3xSD)) on the standard curve.
- The LLOQ as measured here is calculated by interpolating the average background counts (12 wells without analyte) + 10 x standard deviation value (average background counts + (10xSD)) on the standard curve. Alternatively, the true LLOQ can be determined by spiking known concentrations of analyte in the matrix and measuring the percent recovery, and then determining the minimal amount of spiked analyte that can be quantified within a given limit (usually +/- 20% or 30% of the real concentration).
- Read from the standard curve the concentration of analyte contained in the samples.
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Assay Performance Characteristics

AlphaLISA assay performance described below was determined using the 2-step Standard Protocol and AlphaLISA 1X Immunoassay Buffer as assay buffer. The analytes (standards) were prepared in AlphaLISA Immunoassay Buffer, Culture cell medium and FBS. All other components were prepared in AlphaLISA Immunoassay Buffer.

- Assay Sensitivity:

The LDL was calculated as described above. The values correspond to the lowest concentration of analyte that can be detected in a volume of 5 µL sample using the recommended assay conditions.

LDL (pg/mL)	(Analyte diluent)	# of experiments
0.79	Immunoassay Buffer	19
2.78	RPMI + 10%FBS	11
2.21	DMEM + 10%FBS	3
1.24	100% FBS	2

- Assay Precision:

The following assay precision data were calculated from the three independent assays using two different kit lots. In each lot, the analytes were prepared in Immunoassay buffer (IAB), RPMI + 10% FBS, DMEM + 10% FBS and 100% mouse serum. All other components were prepared in AlphaLISA Immunoassay Buffer IAB. Each assay consisted of one standard curve comprising 12 data points (each in triplicate) and 12 background wells (no analytes). The assays were performed in 384-well plate format.

- Intra-assay precision:

The intra-assay precision was determined using a total of 3 independent determinations in triplicate. Shown as CV%.

mouse IL1ß	IAB	RPMI + 10% FBS	DMEM + 10% FBS	Mouse serum
intraCV (%)	3%	3%	3%	2%

- Inter-assay precision:

The inter-assay precision was determined using a total of 3 independent determinations with 9 measurements for 100 p/mL sample. Shown as CV%.

mouse IL1ß	IAB	RPMI + 10% FBS	DMEM + 10% FBS	Mouse serum
interCV (%)	5%	3%	4%	3%

- Spike Recovery:

A known concentration of analyte was spiked into AlphaLISA Immunoassay Buffer IAB, RPMI + 10% FBS, DMEM + 10% FBS and mouse serum. All samples, including non-spiked diluents were measured in the assay. Note that the analytes for the respective standard curves were prepared in IAB, RPMI + 10% FBS, DMEM + 10% FBS and Serum. All other assay components were diluted in IAB.

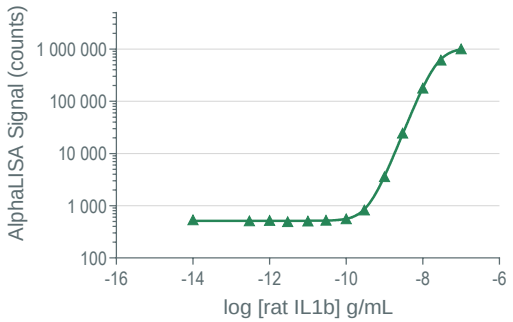
Spiked **mouse IL1ß** (pg/mL)	% Recovery			
	IAB	RPMI + 10% FBS	DMEM + 10% FBS	Mouse serum
100	102%	106%	103%	88%

- Specificity:

Cross-reactivity of the AlphaLISA HP mouse/rat IL1ß Detection Kit was tested using the following proteins at 3 ng/mL in IAB. The cross reactivities were calculated using the signals of 100 ng/mL mouse IL1ß as 100%. No unwanted cross-reactions with related human protein was observed.

Proteins	**Cross Reactivity (%)**
Rat	100%
Human	0%

Please note that to quantitate rat IL1ß from samples it is recommended that a rat recombinant protein (not provided) is used to generate a standard curve for analysis. Below is a typical standard curve generated with rat recombinant protein. An LDL of 136 pg/mL and an LLOQ of 232 pg/mL was observed.



- Calibration

Mouse IL1ß (NIBSC/WHO First International Standard (code 93/668) was tested using the AlphaLISA HP mouse IL1β Detection Kit: 1 unit of Standard NIBSC 93/668 corresponds to 1.5 pg/mL AlphaLISA mIL1ß.

Troubleshooting Guide

You will find detailed recommendations for common situations you might encounter with your AlphaLISA Assay kit at: www.Revvity.com

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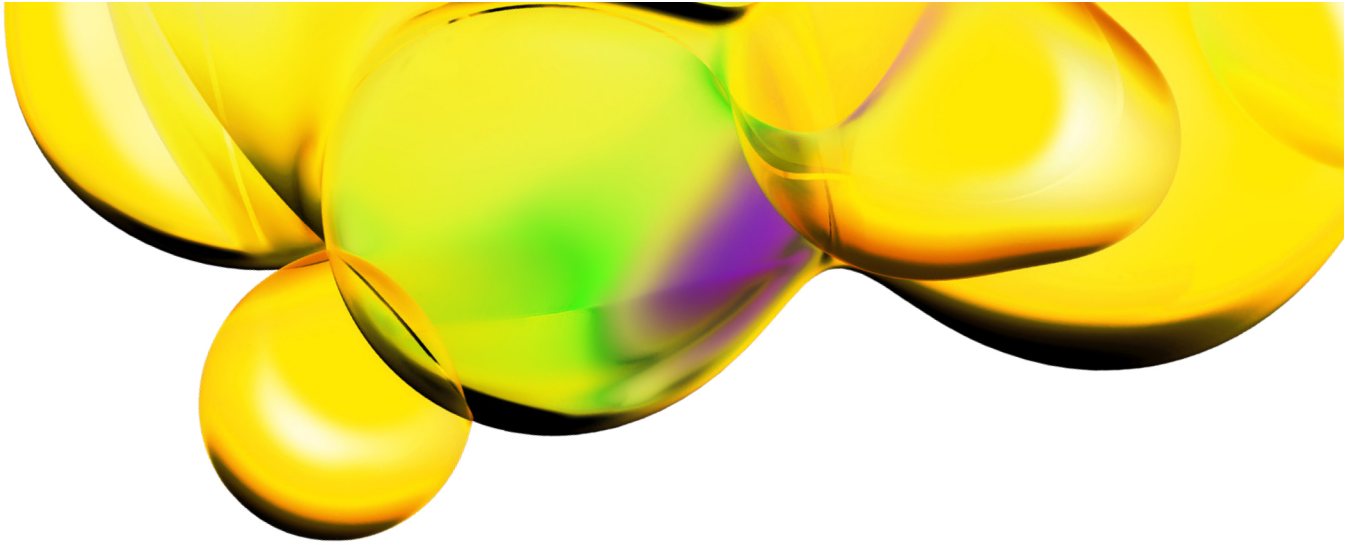

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