MANUAL

AlphaLISA® Mouse TREM2 AlphaLISA Detection Kit

Product number: AL591HV/C/F

Research Use Only. Not for use in diagnostic procedures.

Product Information

Application: This kit is designed for the quantitative determination of mouse TREM2 in buffer, cell culture media and cell supernatant using a homogeneous AlphaLISA assay (no wash steps).

Sensitivity: Lower Detection Limit (LDL): 1.6 pg/mL

Lower Limit of Quantification (LLOQ): 5.5 pg/mL

EC_{50}: 10.4 ng/mL

Dynamic range: 1.6 – 100 000 pg/mL

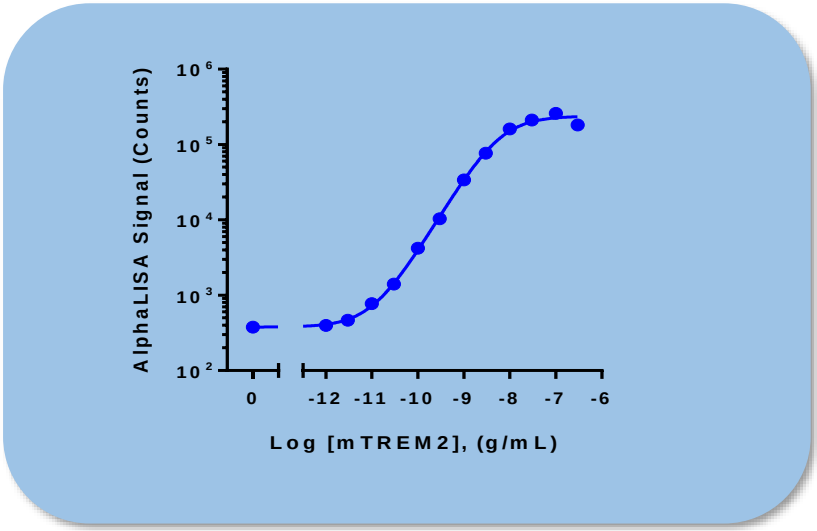


Figure 1. Typical sensitivity curve in AlphaLISA Universal Buffer. The data was generated using a white Optiplate™-384 microplate and the EnVision® Multilabel Plate Reader 2102 with Alpha option.

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

Stability: This kit is stable for at least 6 months from the manufacturing date when stored in its original packaging and the recommended storage conditions.

Analyte of Interest

TREM2 is a transmembrane molecule expressed on myeloid cells. It is known as triggering receptor expressed on myeloid cells 2 (TREM2). It acts as the receptor for a ligand to activate myeloid cells such as dendritic cells, increasing phagocytic activity. Recently, TREM2 has been shown to be involved in neurodegenerative diseases such as ataxia, early dementia and Alzheimer's disease. Elevated levels of TREM2 have been detected in cerebrospinal fluid in Alzheimer's disease models.

Description of the AlphaLISA Assay

AlphaLISA technology allows the detection of molecules of interest in buffer, cell culture media and cell supernatants in a highly sensitive, quantitative, reproducible and user-friendly mode. In this AlphaLISA assay, a biotinylated Anti-mTREM2 Antibody binds to the Streptavidin-coated Alpha Donor beads, while another Anti-mTREM2 Antibody is conjugated to AlphaLISA Acceptor beads. In the presence of the mTREM2, the beads come into close proximity. The excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer in the Acceptor beads, resulting in a sharp peak of light emission at 615 nm (Figure 2).

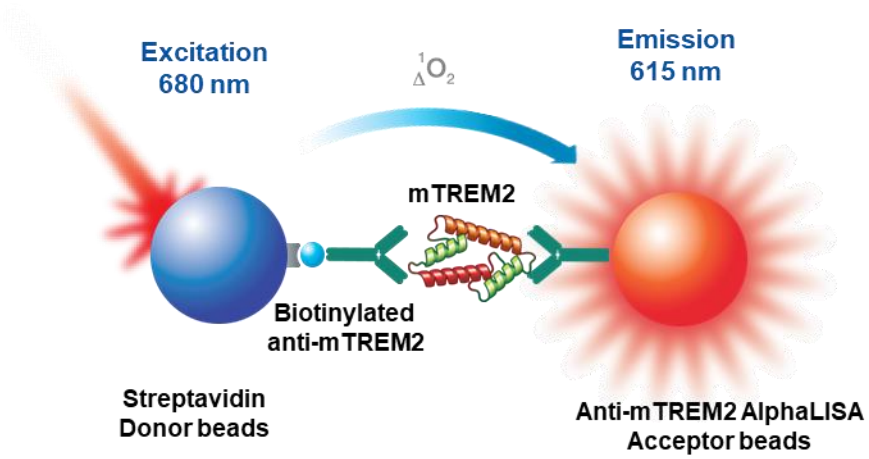


Figure 2. AlphaLISA Mouse TREM2 Assay Principle.

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-Analyte Antibody contains sodium azide. Contact with skin or inhalation should be avoided.

Kit Content: Reagents and Materials

Kit components	AL591HV (100 assay points***)	AL591C (500 assay points***)	AL591F (5000 assay points***)
AlphaLISA Anti-mTREM2 Acceptor beads stored in PBS, 0.05% Kathon CG/ICP, pH 7.2	20 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	80 µL @ 5 mg/mL (1 brown tube, black cap)	200 µL @ 5 mg/mL (1 brown tube, black cap)	2 X 1 mL @ 5 mg/mL (2 brown tubes, black caps)
Biotinylated Anti-mTREM2 Antibody stored in PBS, 0.1% Tween-20, 0.05% NaN_3, pH 7.4	40 µL @ 500 nM (1 tube, black cap)	100 µL @ 500 nM (1 tube, black cap)	1 mL @ 500 nM (1 tube, black cap)
Lyophilized mTREM2 Analyte*	0.3 µg (1 tube, clear cap)	0.3 µg (1 tube, clear cap)	0.3 µg (1 tube, clear cap)
AlphaLISA Immunoassay Buffer (10X)**	2 mL, 1 small bottle	10 mL, 1 medium 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 90 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 # AL591S).

** 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-mTREM2 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 (2000g, 10-15 sec). Re-suspend all reagents by vortexing before use.
- Use Milli-Q® grade H_2O to dilute 10X AlphaLISA Universal 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: D6 as, 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 manual described below is an example for generating one standard curve in a 50 µL final assay volume (48 wells, triplicate determinations). The manuals 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 manual 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**	**AlphaLISA Acceptor Beads**	**Biotinylated Antibody**	**SA-Donor beads**	**Plate recommendation**
AL591HV	100	100 µL	10 µL	20 µL	20 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
AL591C	250	100 µL	10 µL	20 µL	20 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
	500	50 µL	5 µL	10 µL	10 µL	25 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate™-384 (cat # 6005350)
	1 250	20 µL	2 µL	4 µL	4 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	2 µL	2 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)
AL591F	5 000	50 µL	5 µL	10 µL	10 µL	25 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate-384 (cat # 6005350)
	12 500	20 µL	2 µL	4 µL	4 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	2 µL	2 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)

3 Step Manual 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 Universal Buffer:
Add 5 mL of 10X AlphaLISA Immunoassay Buffer to 45 mL Milli-Q® grade H_2O.

2) Preparation of mTREM2 analyte standard dilutions:
   a. Reconstitute lyophilized mTREM2 (0.3 µg) in 100 µL Milli-Q® grade H_2O.
   b. Prepare standard dilutions as follows in 1X AlphaLISA Immunoassay Buffer (change tip between each standard dilution):

Tube	Vol. of mTREM2 (µL)	Vol. of diluent (µL) *	[mTREM2] in standard curve	
			(g/mL in 5 µL)	(pg/mL in 5 µL)
A	10 µL reconstituted mTREM2	90	3.0E-07	300 000
B	60 µL of tube A	120	1.0E-07	100 000
C	60 µL of tube B	140	3.0E-08	30 000
D	60 µL of tube C	120	1.0E-08	10 000
E	60 µL of tube D	140	3.0E-09	3 000
F	60 µL of tube E	120	1.0E-09	1 000
G	60 µL of tube F	140	3.0E-10	300
H	60 µL of tube G	120	1.0E-10	100
I	60 µL of tube H	140	3.0E-11	30
J	60 µL of tube I	120	1.0E-11	10
K	60 µL of tube J	140	3.0E-12	3
L	60 µL of tube K	120	1.0E-12	1
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 Universal Buffer, cell culture media or serum). The diluent used to dilute standards should match the sample type as closely as possible. We recommend preparing cell lysate samples and analyte dilutions used for analysis of cell lysate samples in 1X AlphaLISA lysis buffer (supplied in the kit as a 5X solution).

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.

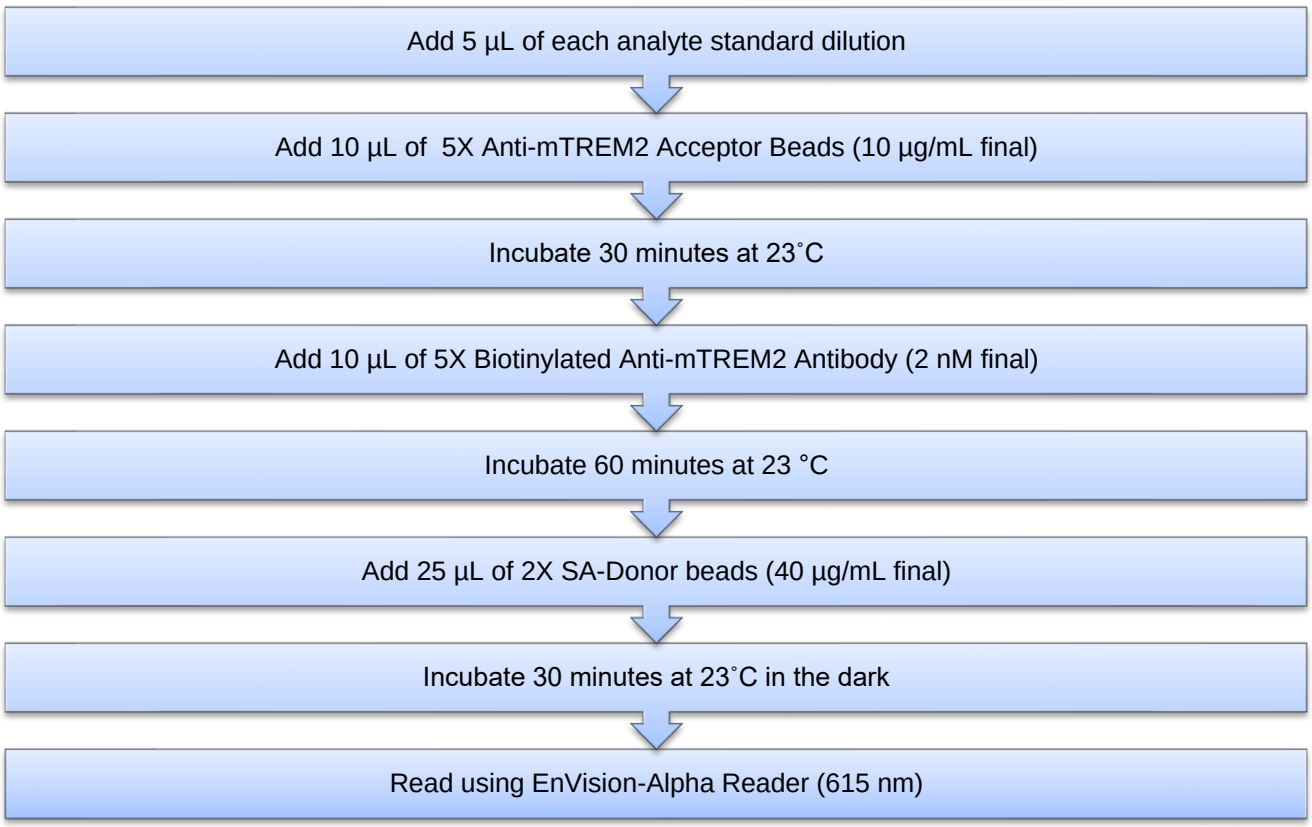
** Four background points in triplicate (12 wells) are used when LDL is calculated. If LDL does not need to be calculated, one background point in triplicate can be used (3 wells).

3) Preparation of 5X Anti-mTREM2 AlphaLISA Acceptor beads (50 µg/mL):
   a. Prepare just before use.
   b. Add 50 µL Anti-mTREM2 Acceptor beads to 4950 µl of 1X AlphaLISA Immunoassay Buffer. Mix briefly.

4) Preparation of 5X biotinylated Anti-mTREM2 antibody (10 nM):
   a. Prepare just before use.
   b. Add 100 µL 500 nM Biotinylated Anti-mTREM2 Antibody to 4900 µl of 1X AlphaLISA Immunoassay Buffer.

5) 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.

6) In a white Optiplate (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 3-step manual using AlphaLISA Immunoassay Buffer (IAB) as assay buffer. The analytes (standards) were prepared in IAB, DMEM, RPMI, DMEM + 10% FBS and RPMI + 10% FBS and all other components were prepared in IAB.

- 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
1.6	IAB	8
4.6	DMEM	6
6.5	RPMI	6
3.2	DMEM + 10% FBS	6
3.9	RPMI + 10% FBS	6

- 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 IAB, DMEM, RPMI, DMEM + 10% FBS and RPMI + 10% FBS. All other components were prepared in 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 format.

- Intra-assay precision:

The intra-assay precision was determined using a total of 16 independent determinations in triplicate. Shown as CV%.

mTREM2	IAB	DMEM	RPMI	DMEM + 10% FBS	RPMI + 10% FBS
CV (%)	6	6	7	5	11

- Inter-assay precision:

The inter-assay precision was determined using a total of 3 independent determinations with 9 measurements for 1 ng/mL sample. Shown as CV%.

mTREM2	IAB	DMEM	RPMI	DMEM + 10% FBS	RPMI + 10% FBS
CV (%)	9	13	17	3	15

- Spike Recovery:

Three known concentrations of analyte were spiked into IAB, DMEM, RPMI, DMEM + 10% FBS and RPMI + 10% FBS. All samples, including non-spiked diluents were measured in the assay. Note that the analytes for the respective standard curves were prepared in IAB, DMEM, RPMI, DMEM + 10% FBS and RPMI + 10% FBS. All other assay components were diluted in IAB.

Spiked mTREM2 (ng/mL)	% Recovery				
	IAB	DMEM	RPMI	DMEM + 10% FBS	RPMI + 10% FBS
10	75	77	73	101	92
3	92	84	78	98	100
1	99	78	78	101	111

- Specificity:

Cross-reactivity of the mTREM2 AlphaLISA Detection Kit was tested using the following proteins at 10 ng/mL in IAB. The cross reactivities were calculated using the signals of 10 ng/mL mTREM2 as 100%.

Tested Proteins	% Cross Reactivity
Human TREM2	1.1

- Mouse Raw 264.7 Cell Supernatant Experiments

Mouse Cell Line RAW 264.7 cells (1 x 10^5 cells/mL) were cultured in DMEM supplemented with 10% FBS for 24 hours. Aliquots of the cell culture supernatants were removed and assayed for levels of mTREM2.

The cell culture supernatant samples were tested, and the assay was performed along with a standard curve prepared in DMEM + 10% FBS. All other components of the assay were prepared in AlphaLISA Immunoassay Buffer. Concentrations of mTREM2 in cell culture supernatant were determined by interpolating to the standard curve. In RAW 264.7 cell supernatant, 4.1 ng/mL mTREM2 was detected (average of three experiments).

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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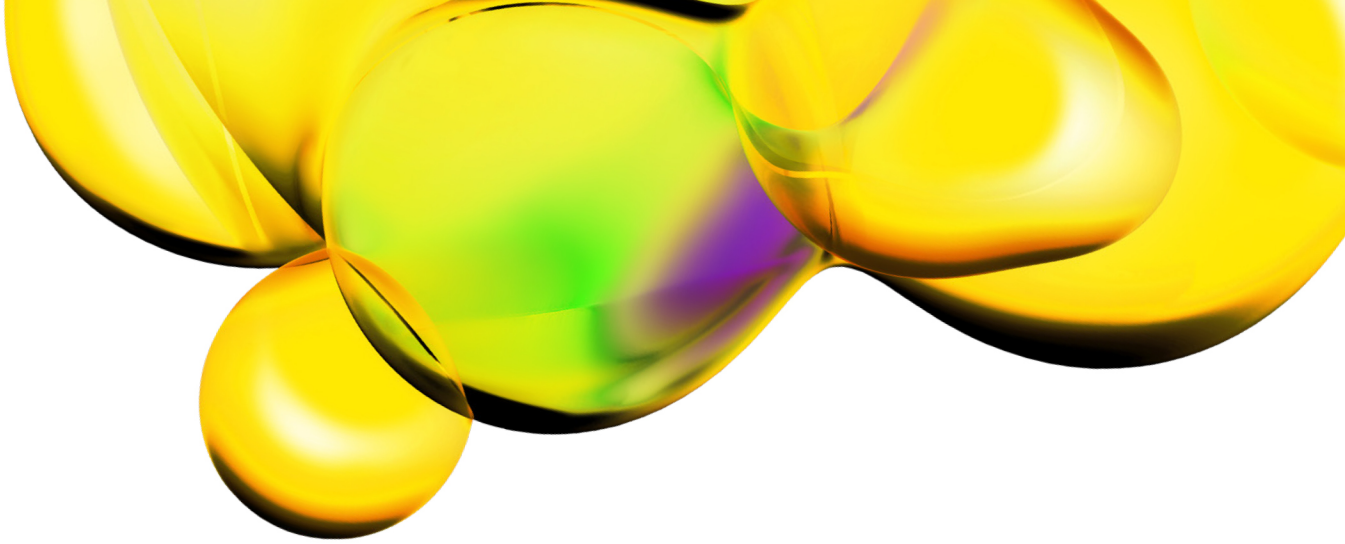

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