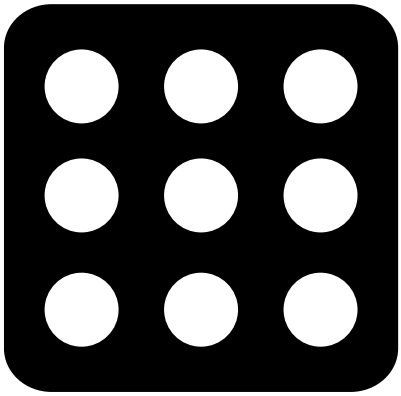
Datasheet & Protocol

EYRAplex Flex:
Human Cytokine/Chemokine, pre-mix

EYX-HCC-PM
Platform: Flow Cytometry



Watch video tutorial

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Introduction

EYRAplex Flex is a custom magnetic bead-based multiplex assay for the simultaneous detection and quantification of multiple analytes in a single sample. The magnetic beads have been dyed with different concentrations of two fluorophores, resulting in distinct bead populations with unique bead IDs. Each bead population has been conjugated with a specific monoclonal antibody, allowing specific binding to its corresponding analyte. Multiplex analysis is enabled by combining several bead populations that are supplied as a capture bead mix. Please see the included Certificate of Analysis for your kit's panel-specific details.

In EYRAplex, the capture bead mix is added to wells in a 96-well plate. Samples and standards are then added. During incubation, the capture antibodies bind to analytes present in the sample. Detection occurs in two steps. First, a biotin-labeled monoclonal antibody mix (detection mAb mix) is added. These detection antibodies bind to the captured analytes. Streptavidin-PE is then added, binding to the biotin and generating a fluorescent signal proportional to the analyte concentration.

The fluorescent signal from each bead ID determines the analyte, while the streptavidin-PE signal reflects its quantity. Analytes are quantified by comparing the signals to standard curves.

Contents

The kit includes	Dilution	Quantity	Notes
Capture bead mix: Human Cytokine/Chemokine	-	6 ml	The included Capture bead mix corresponds to the selected analytes in the customized panel.
Detection mAb mix: Human	1:20	300 µl	H2A or H3A
Standard mix C: EYRAplex, human	See standard datasheet	1	
Streptavidin-PE	1:100	60 µl	
Assay diluent: EYRAplex	-	2 x 20 ml	
Streptavidin-PE diluent	-	6 ml	
Wash buffer concentrate	1:20	120 ml	
96-well plate (black)	-	1	
Adhesive plate covers	-	4	
Black plate lid	-	1	
Flow Setup EYRAbeads	-	900 µl	See Preparations

To ensure total recovery of the stated quantity, vials and bottles have been overfilled.

Shipping and storage

- Shipped at ambient temperature.
- Store at 4-8 °C upon receipt.
- The expiry date indicates how long unopened products, stored according to instructions, are recommended for use.

Materials required but not supplied

- 96-well plate washer for magnetic beads (hand-held or automated)
- Distilled or deionized water
- Vortex mixer
- Orbital shaker for microtiter plates
- Tubes or plates for dilutions
- Syringe and filter
- Precision pipettes and tips

Safety information

Human and animal samples should be treated as potentially hazardous biological materials. All materials should be disposed of in accordance with local regulations. For detailed safety information regarding the reagents included in this kit, please consult the Safety Data Sheet (SDS).

Guidelines

Orbital shaker
We recommend using a microtiter plate orbital shaker with a 3 mm (0.12 in) orbit at 800 RPM.

Plate washer
Both automated and hand-held magnetic bead washers for 96-well plates are suitable. Refer to the manufacturer's manual for automated washer setup instructions.

We recommend the following washers and settings:

- **Automated: BioTek 50 TS (with Flat Magnet for 96-well plates, 7103016, Agilent)**. Use the plate format Plate and make sure to prime before wash (volume: 5, flow rate: 5). Start with one cycle of soak for 1 min 30 s without shaking. Perform four cycles of wash incorporating aspiration (Travel rate: 6, Delay: 0, Z-offset: 38, Y-offset: -20), dispensing (Volume: 200, Flow rate: 7, Z-offset: 120, Y-offset: 0) and soak (45 s, no shaking). Finish with a final aspiration (Travel rate: 6, Delay: 0, Z-offset: 35, Y-offset: -20).
- **Hand-held: VP 771HH-G-4 or LifeSep 96F**. Place the plate on the magnetic rack and secure it properly. Let the beads initially settle for 60 s. Decant the plate while attached to the magnetic rack and gently tap it on a clean paper towel, add 200 µl wash buffer and let the beads settle for 30 s. Repeat three times (four washes in total). End by decanting and gently tapping the plate.

Plate washing
- Each wash step consists of four washes with 200 µl of Wash buffer per well.
- For automated washers, residual Wash buffer will remain in each well, do not remove it.
- After each wash step (four washes), remove the plate from the washer magnet as soon as possible to prevent bead aggregation.

Magnetic beads
Magnetic beads settle quickly. Vortex the Capture bead mix immediately before use and briefly at regular intervals (at least every 30 seconds) while adding to the plate. The Capture bead mix may also be vortexed and poured into a reagent reservoir before pipetting. Use a multichannel pipette and pipette the bead solution up and down regularly to maintain suspension.

Standard mix
Once reconstituted, use immediately. Do not freeze for future use.

Assay diluent
Assay diluent: EYRAplex is used for dilution of standards, samples, the Detection mAb mix, and as reading buffer. Remove Assay diluent: EYRAplex from the refrigerator just before use and return it immediately afterward. Do not leave Assay diluent: EYRAplex at room temperature as it may cause bead aggregation.

Plate handling
Keep the plate clean and dry throughout the assay, and avoid touching the underside. Do not write on the adhesive plate cover.

Light protection
The magnetic capture beads and Streptavidin-PE are light-sensitive. Use the black plate lid during incubations, placed over the adhesive plate cover, to protect from light exposure.

Flow cytometer requirements

EYRAplex assays use **APC- and APC-Cy7-equivalent dyes** for bead identification and PE to quantify captured analytes. Flow cytometers must be equipped with a **red laser (635 nm)** and **blue laser (488 nm)*** as well as filters to detect PE (575/25 nm), APC-equivalent (660/20 nm), and APC-Cy7-equivalent (780/60 nm).

*Note: *Green (532 nm) and yellow-green (561 nm) lasers may also be suitable for PE excitation, but EYRAplex performance has not been evaluated by Mabtech using these lasers.*

Preparations

Standard

Reconstitute the lyophilized standards and dilute to create the standard curve according to the EYRAplex standard datasheet provided with the kit.

Wash buffer

Dilute 50 ml of Wash buffer concentrate in 950 ml of distilled or deionized water. If crystals are present, bring the solution to room temperature and gently mix until fully dissolved.

Samples

After collection, centrifuge all samples to remove cells and debris. For **serum and plasma**, centrifuge at 1,500–2,000 × g for 10–15 minutes, and carefully transfer the supernatant. For **cell culture supernatants**, an initial centrifugation at 300–500 × g for 5–10 minutes is recommended to remove cell debris, followed by a secondary spin at 1,000–2,000 × g if further clarification is needed. Following centrifugation, store the samples frozen in aliquots at -80 °C if not used immediately. Do not use samples stored at +4-8 °C, as this may lead to bead aggregation during the assay. Upon thawing, samples should be centrifuged again at 10,000 × g for 5–10 minutes to remove any precipitates or residual debris that may have formed during storage.

- **Serum and plasma:** Dilute the samples at least 4-fold in Assay diluent: EYRAplex to ensure optimal assay performance. Perform the dilutions in tubes or plates, and always add Assay diluent: EYRAplex before adding the sample. **IMPORTANT:** Allow the diluted sample to incubate for a minimum of 20 minutes at room temperature before adding it to the beads. Strongly hemolyzed or lipemic samples may lead to inaccurate results. If analyte concentrations exceed the assay's upper limit of quantification (ULOQ), additional dilution will be necessary.

- **Cell supernatants:** May be used undiluted or diluted in Assay diluent: EYRAplex, depending on expected analyte concentrations.

Note: Be sure to include an appropriate positive control sample (e.g. supernatant from PBMCs stimulated with PHA, LPS, or peptides).

Detection mAb mix

Dilute the Detection mAb mix in Assay diluent: EYRAplex according to the contents table and filter through a 0.2 µm filter (25 mm diameter recommended). This preparation should be done no more than 15 minutes before adding to the plate.

Streptavidin-PE

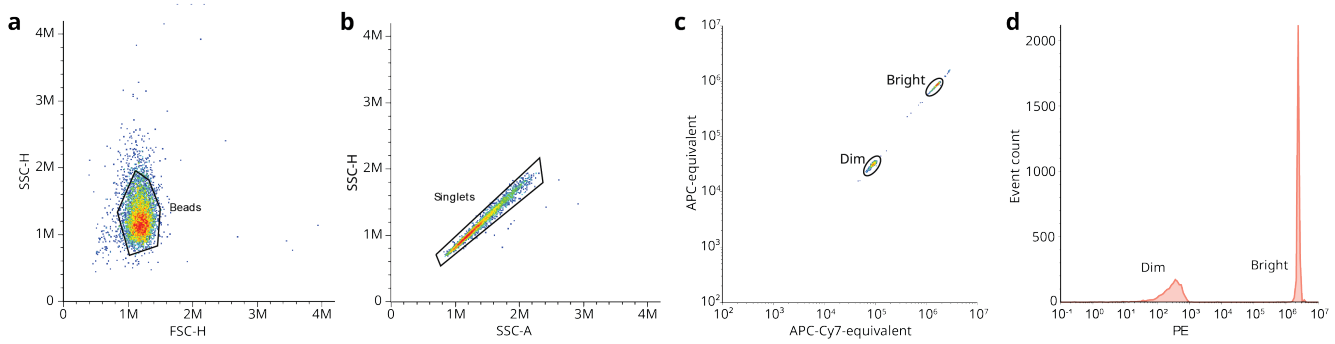
Dilute Streptavidin-PE 1:100 in Streptavidin-PE diluent no more than 15 minutes before adding to the plate.
Note: Streptavidin-PE is light-sensitive; protect the vial with aluminum foil.

Flow cytometer setup

The included Flow Setup EYRAbeads should be used to ensure the setup of your flow cytometer allows for proper acquisition of the EYRAplex assay. This can be done at any point prior to data acquisition. The Flow Setup EYRAbeads consists of a Bright and Dim dyed bead population, simulating the highest and lowest fluorescent signals generated in the respective fluorescent emission channels.

1. **Start the flow cytometer** according to the manufacturer's instructions and internal SOPs, including any warm-up, priming, and daily quality control steps.
2. **Prepare Flow Setup EYRAbeads**
    a. Vortex the Flow Setup EYRAbeads vial for 30 seconds and add **300-400 µl** to an appropriate FACS tube compatible with your flow cytometer.
    Note: the vial contains 900 µl of the Flow Setup EYRAbeads, allowing for 2-3 instrument setups
3. **Set FSC and SSC**
    a. Open a new experiment, protocol, or instrument setup module.
    b. Create a dot plot with FSC-H on the x-axis and SSC-H on the y-axis (**Fig 1a**).
    c. Acquire the Flow Setup EYRAbeads at a low flow rate and adjust FSC and SSC voltage/gain until the bead population is visible and completely on scale. Increase the flow rate if needed.
    d. Draw a "**Beads**" gate around the main population, excluding debris.
    Note: You can pause acquisition to conserve beads when drawing gates.
    e. From the Beads gate, create a dot plot with SSC-A on the x-axis and SSC-H on the y-axis, both on a linear scale and draw a "**Singlets**" gate (**Fig 1b**).
4. **Set APC and APC-Cy7 emission channels**
    a. From the Singlets gate, create a dot plot and set APC-Cy7 on the x-axis and APC on the y-axis, both on a log scale (**Fig 1c**).
    b. Adjust the voltages/gains for APC and APC-Cy7 until the two Flow Setup EYRAbeads populations are clearly visible and on scale.
    Note: Both populations must be fully on scale, with Dim in the bottom-left region and Bright in the top-right region of the plot.
5. **Set PE emission channel**
    a. From the Singlets gate, create a PE histogram setting the x-axis to the PE channel on log scale and y-axis to Count (**Fig 1d**).
    b. Adjust the voltage/gain until two PE peaks are simultaneously visible and on scale.
    Note: Both PE peaks must be visible and on scale. If either peak is off scale, there is a risk your samples will be off scale, leading to erroneous MFI values and incorrect quantification.
6. **Save the instrument settings.**

Figure 1: gating instructions for instrument setup



Protocol

Prepare reagents, standards, and samples as described in the Preparations section.

1. **Capture beads**
   Vortex the Capture bead mix for 30 seconds and immediately add 50 µl per well.
   Note: Beads settle quickly. Vortex regularly (at least every 30 seconds) during dispensing.

2. **Wash** the plate 4 times with 200 µl of diluted Wash buffer per well.
   Note: See Guidelines and Preparations.

3. **Standards and samples**
   Add 50 µl per well of standard and samples diluted in Assay diluent: EYRAplex. Also include wells with 50 µl of Assay diluent: EYRAplex without standard, which will serve as assay background controls (blank wells). Cover the plate with an adhesive plate cover and the black plate lid to protect from light and incubate for 2 hours at room temperature, 800 RPM, on an orbital shaker. Alternatively, incubation can be done overnight at 4–8 °C with shaking at 800 RPM.

4. **Wash** the plate 4 times as described above.

5. **Detection mAb mix**
   Add 50 µl per well of the diluted and filtered Detection mAb mix. Cover the plate with a new adhesive plate cover and the black plate lid to protect from light and incubate for 1 hour at room temperature, 800 RPM, on an orbital shaker.

6. **Wash** the plate 4 times as described above.

7. **Streptavidin-PE**
   Add 50 µl per well of the diluted Streptavidin-PE. Cover the plate with a new adhesive plate cover and the black plate lid to protect from light and incubate for 30 minutes at room temperature, 800 RPM, on an orbital shaker.

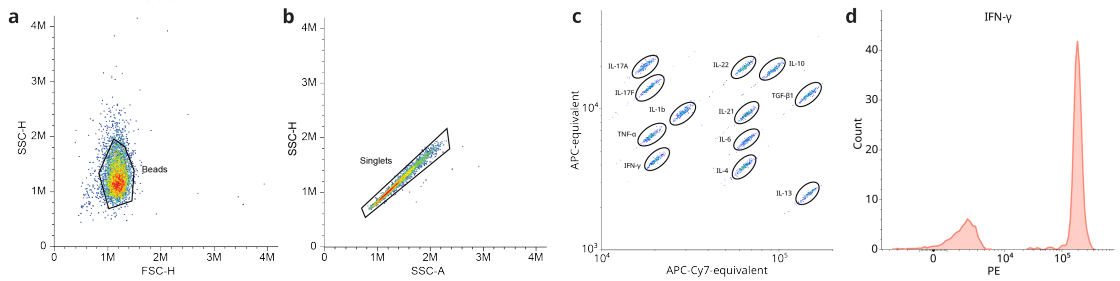
8. **Wash** the plate 4 times as described above.

9. **Assay diluent**
   Add 100-150 µl of Assay diluent: EYRAplex, PBS, or appropriate FACS buffer per well and acquire on a flow cytometer as outlined in the next section.
   Note: Alternatively, the plate may be stored with the Assay diluent: EYRAplex overnight at 4-8 °C, protected from light.

Data acquisition

Load the instrument settings saved during flow cytometer setup (see **Preparations**).

1. **Confirm instrument settings and set gates**
    a. Transfer 10–20 µl from the S1 and Blank wells to a single FACS tube and acquire at a low flow rate. This combines the highest and lowest PE signals in one acquisition so all populations can be confirmed on scale.
    b. Draw the gates as outlined below in **Fig 2a-d** using the specific Bead IDs outlined for your kit and confirm that all Bead IDs and PE peaks are on scale in the APC, APC-Cy7, and PE channels.
    Figure 2: gating instructions for data acquisition



2. **Acquire standards and samples**
    a. Once gating is complete, set acquisition to a minimum of 400 events per Bead ID gate and acquire all standards and samples at an appropriate flow rate for your instrument.
    b. Save the .fcs files, being sure to include the **well ID** in the file name.

3. **Export PE MFI values**
    a. PE median fluorescence intensity (MFI) values from each Bead ID can be exported directly from the flow cytometer software to an Excel or CSV file, or analyzed in external flow cytometry software using the gating strategy above before export.
    Note: To import data into Mabtech Opal, the well ID must be included in each FCS file name (e.g., Standard_1_Well_A1_Plate_1.fcs), with one well per row and the corresponding analyte MFI values in subsequent columns.

- PE MFI values organized into columns
- One analyte per column

- One sample per row
- Well-ID must be present in name

	A	B	C	D	E
1	File	IL-17A	IL-17F	IL-8	TNFa
2	plate 1 sample_A1 c0.fcs	775.21343	696.17260	772.14660	581.09643
3	plate 1 sample_A2 c1.fcs	20162.660	12119.454	59458.843	55719.320
4	plate 1 sample_A3 c2.fcs	645.56976	635.11462	603.88861	504.35574
5	plate 1 sample_A4 c3.fcs	4542.5473	20847.400	150297.31	8374.0761
6	plate 1 sample_A5 c4.fcs	163985.31	526658.93	2098666.5	1130261.6
7	plate 1 sample_A6 c5.fcs	655.74609	847.42358	1809363.3	9290.3642
8	plate 1 sample_A7 c6.fcs	3968.2814	4174.0385	393149.68	3407.9560

4. **Data analysis in Mabtech Opal**
    a. Open the PE MFI values Excel or CSV export file in Mabtech Opal.
    b. In the Layout tab, define the plate layout denoting standards, samples, replicates, and dilution factors.
    c. Enter the S1 concentration for each analyte described in the **Standard Mix datasheet**. Opal will fit the standard curves and calculate concentrations for all unknown samples automatically.

Performance

Precision

Analyte	Intra-assay CV(%)	Inter-assay CV(%)
CCL2 (MCP-1)	4	9
CCL3 (MIP-1α)	3	11
CCL4 (MIP-1β)	4.0	12
CCL11 (Eotaxin)	3	8
CCL22 (MDC)	3	11
CD25 (IL-2Rα)	3	8
CXCL12	4	10
GM-CSF	3	10
IFN-α pan	5	10
IFN-γ	3	9
IL-1α	3	10
IL-1β	4	9
IL-2	4	11
IL-4	2	10
IL-5	5	12
IL-6	4	9

Analyte	Intra-assay CV(%)	Inter-assay CV(%)
IL-7	3	10
IL-8 (CXCL8)	3	10
IL-10	3	11
IL-12 (p70)	3	11
IL-13	2	9
IL-17A	3	9
IL-17F	3	10
IL-18	3	10
IL-22	3	11
IL-29 (IFN-λ1)	4	11
IL-31	3	10
IP-10 (CXCL10)	3	9
Perforin	3	8
TGF-β1 (Latent TGF-β1)	5	12
TNF-α	4	10

Accuracy

	Average recovery (%) in			
Analyte	Heparin plasma	Citrate plasma	EDTA plasma	Serum
CCL2 (MCP-1)	66	75	71	96
CCL3 (MIP-1α)	81	89	93	80
CCL4 (MIP-1β)	98	82	76	131
CCL11 (Eotaxin)	59	85	89	81
CCL22 (MDC)	97	100	91	131
CD25 (IL-2Rα)	56	63	59	*

	Average recovery (%) in			
Analyte	**Heparin plasma**	**Citrate plasma**	**EDTA plasma**	**Serum**
CXCL12	*	*	*	*
GM-CSF	73	77	78	83
IFN-α pan	74	77	77	94
IFN-γ	72	73	78	80
IL-1α	*	*	*	*
IL-1β	89	96	106	87
IL-2	88	89	95	85
IL-4	86	76	74	120
IL-5	90	91	91	96
IL-6	83	87	88	91
IL-7	*	77	82	94
IL-8 (CXCL8)	90	92	93	103
IL-10	75	80	82	78
IL-12 (p70)	57	82	84	73
IL-13	92	87	91	95
IL-17A	70	81	79	75
IL-17F	87	89	87	120
IL-18	47	59	58	*
IL-22	78	82	84	88
IL-29 (IFN-λ1)	57	72	74	76
IL-31	74	69	71	67
IP-10 (CXCL10)	95	71	92	*
Perforin	27	*	*	*
TGF-β1 (Latent TGF-β1)	59	63	55	66
TNF-α	60	79	80	69

Spike recovery was assessed in different matrices from healthy donors using the assay standard at three concentrations (high, medium, low) and unspiked controls, with three to four replicates per condition. Several independent experiments were performed, and the average recovery was calculated relative to spiked Assay diluent: EYRAplex. Data was obtained from analysis of first batches.
*See Linearity

Linearity

	Average recovery (%) in			
Analyte	**Heparin plasma**	**Citrate plasma**	**EDTA plasma**	**Serum**
CD25 (IL-2Rα)	**	**	**	76 (1:16 - 1:128)
CXCL12	108 (1:16 - 1:512)	115 (1:8 - 1:512)	114 (1:4 - 1:512)	94 (1:4 - 1:512)
IL-1α	86 (1:64 - 1:512)	87 (1:16 - 1:512)	87 (1:16 - 1:512)	73 (1:8 - 1:128)
IL-18	**	**	**	79 (1:16 - 1:128)
IP-10 (CXCL10)	**	**	**	93 (1:4 - 1:128)
Perforin	94 (1:16 - 1:512)	89 (1:16 - 1:512)	87 (1:16 - 1:512)	83 (1:32 - 1:512)

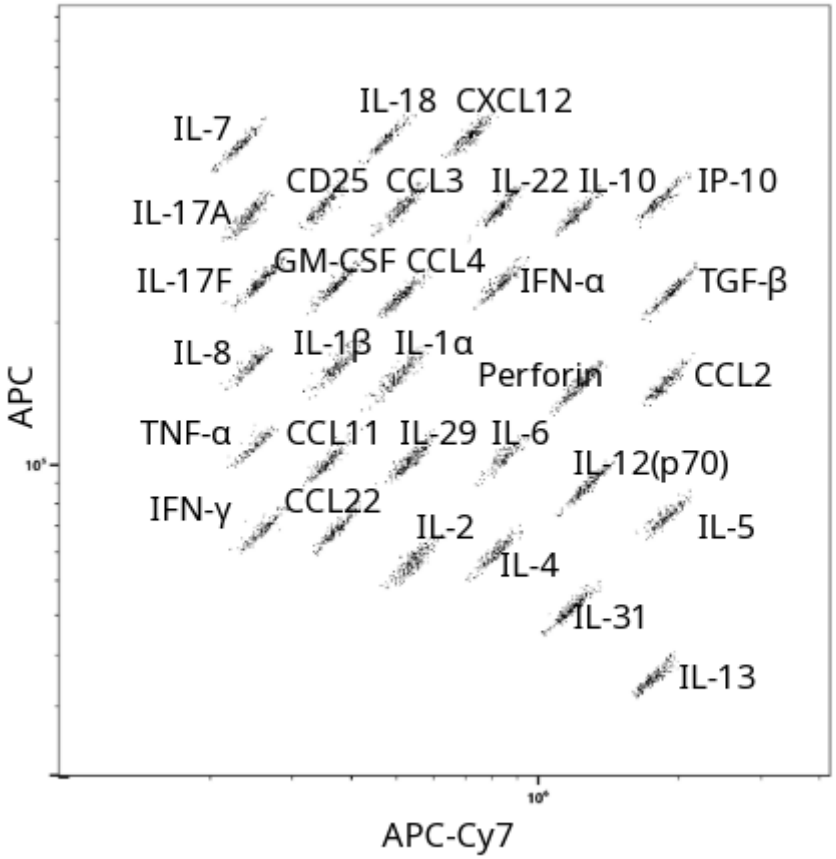
Data was obtained from the first batch analysis.
**See Accuracy

Bead ID

Bead ID

Analyte	Bead ID
CCL2 (MCP-1)	A6
CCL3 (MIP-1α)	D4
CCL4 (MIP-1β)	D5
CCL11 (Eotaxin)	E7
CCL22 (MDC)	E8
CD25 (IL-2Rα)	E4
CXCL12	C3
GM-CSF	E5
IFN-α pan	C5
IFN-γ	F8
IL-1α	D6
IL-1β	E6
IL-2	D8
IL-4	C8
IL-5	A7
IL-6	C7

Analyte	Bead ID
IL-7	F3
IL-8 (CXCL8)	F6
IL-10	B4
IL-12 (p70)	B7
IL-13	A8
IL-17A	F4
IL-17F	F5
IL-18	D3
IL-22	C4
IL-29 (IFN-λ1)	D7
IL-31	B8
IP-10 (CXCL10)	A4
Perforin	B6
TGF-β1 (Latent TGF-β1)	A5
TNF-α	F7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CCL3
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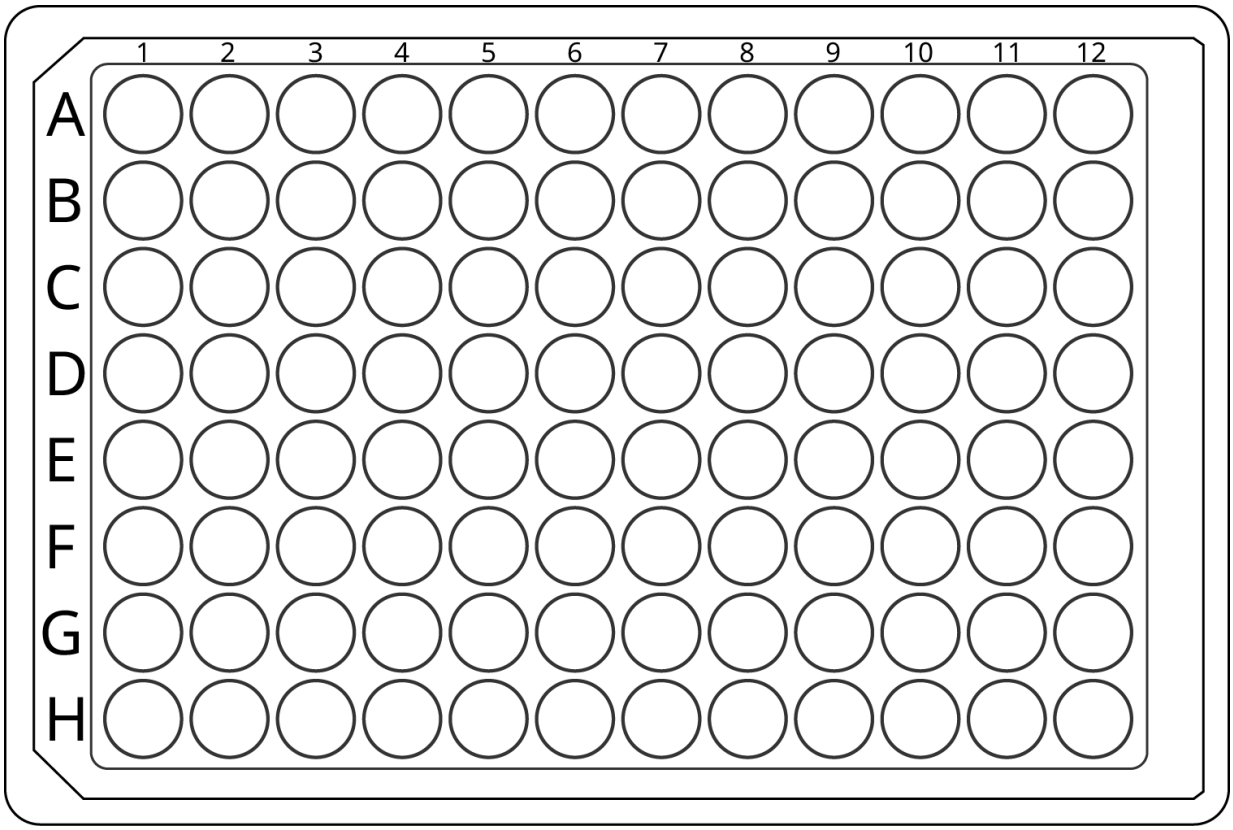
GM-CSF
CCL4
IL-17F
IFN-α
TGF-β
IL-8
IL-1β
IL-1α
Perforin
CCL2
TNF-α
CCL11
IL-29
IL-6
IL-12(p70)
IFN-γ
CCL22
IL-2
IL-5
IL-4
IL-31
IL-13
APC
APC-Cy7



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