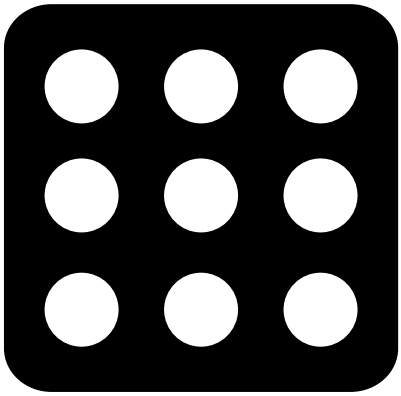
Datasheet & Protocol

EYRAplex: Mouse Cytokine/Chemokine (30-plex)

5545-1EY-1
Platform: Mabtech EYRA



Watch video tutorial

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Intended use

EYRAplex: Mouse Cytokine/Chemokine (30-plex) is intended for the quantification of mouse CCL2 (MCP-1), CCL5 (RANTES), CCL11 (Eotaxin), CXCL1 (KC/GRO-α), CXCL2 (MIP-2), CXCL5 (LIX), CXCL9 (MIG), G-CSF, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12 (p70), IL-12/-23 (p40), IL-13, IL-15, IL-17A, IP-10 (CXCL10), LIF, M-CSF, TNF-α, and VEGF-A in mouse serum and cell culture supernatants. The assay is designed for analysis using Mabtech EYRA™, but can also be used in flow cytometry. For research use only. Not for use in diagnostic procedures.

Introduction

EYRAplex is a 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.

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
Capture bead mix: Mouse Cytokine/Chemokine (30-plex)	-	6 ml
Detection mAb mix: Mouse M5A	1:20	300 µl
Standard mix K: EYRAplex, mouse	See Standard datasheet	1
Streptavidin-PE	1:100	60 µl
Assay diluent: EYRAplex, mouse	-	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

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

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. When transferring the plate to Mabtech EYRA™, handle it carefully to prevent disrupting the settled beads. Remove the black plate lid before placing the plate in the instrument for analysis.

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.

Incubation

The recommended sample/standard incubation time is two hours at room temperature, as stated in the protocol. Alternatively, incubation can be done overnight at 4–8 °C with shaking at 800 RPM.

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**, 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:** 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.

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.

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.

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 50 µl of Assay diluent: EYRAplex per well and proceed to step 10.
   Note: Alternatively, the plate may be stored with the Assay diluent: EYRAplex overnight at 4-8 °C, protected from light.

10. **Analysis**
    For Mabtech EYRA™ analysis: Cover the plate with a new adhesive plate cover and the black plate lid to protect from light. Place the plate on an orbital shaker at 800 RPM for 5 minutes. Then, allow the beads to settle for 20 minutes. Remove the black plate lid and insert the plate into Mabtech EYRA™. The adhesive plate cover may remain in place during reading.

Performance

Precision

Analyte	Intra-assay CV(%)	Inter-assay CV(%)
CCL2 (MCP-1)	3	7
CCL5 (RANTES)	4	7
CCL11 (Eotaxin)	4	7
CXCL1 (KC/GRO-α)	3	9
CXCL2 (MIP-2)	4	10
CXCL5 (LIX)	3	9
CXCL9 (MIG)	4	5
G-CSF	4	4
GM-CSF	3	10
IFN-γ	2	8
IL-1α	3	6
IL-1β	2	9
IL-2	3	11
IL-3	4	4
IL-4	3	8

Analyte	Intra-assay CV(%)	Inter-assay CV(%)
IL-5	3	8
IL-6	3	8
IL-7	4	10
IL-9	4	5
IL-10	3	5
IL-12 (p70)	4	7
IL-12/-23 (p40)	3	6
IL-13	3	5
IL-15	3	7
IL-17A	3	6
IP-10 (CXCL10)	4	8
LIF	2	5
M-CSF	3	7
TNF-α	3	7
VEGF-A	3	6

Intra-assay and inter-assay precision were evaluated using the assay standard at three concentrations (high, medium, low) in Assay diluent: EYRAplex, mouse. CV values are based on several independent experiments, using multiple replicates per condition. Data was obtained from the first batch analysis.

Accuracy

	Average recovery (%) in
Analyte	**Serum**
CCL2 (MCP-1)	121
CCL5 (RANTES)	62
CCL11 (Eotaxin)	71
CXCL1 (KC/GRO-α)	98
CXCL2 (MIP-2)	61
CXCL5 (LIX)	*

	Average recovery (%) in
Analyte	**Serum**
CXCL9 (MIG)	137
G-CSF	72
GM-CSF	89
IFN-γ	81
IL-1α	83
IL-1β	77
IL-2	92
IL-3	101
IL-4	*
IL-5	94
IL-6	79
IL-7	100
IL-9	48
IL-10	92
IL-12 (p70)	93
IL-12/-23 (p40)	97
IL-13	34
IL-15	114
IL-17A	98
IP-10 (CXCL10)	94
LIF	43
M-CSF	*
TNF-α	72
VEGF-A	74

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

Linearity

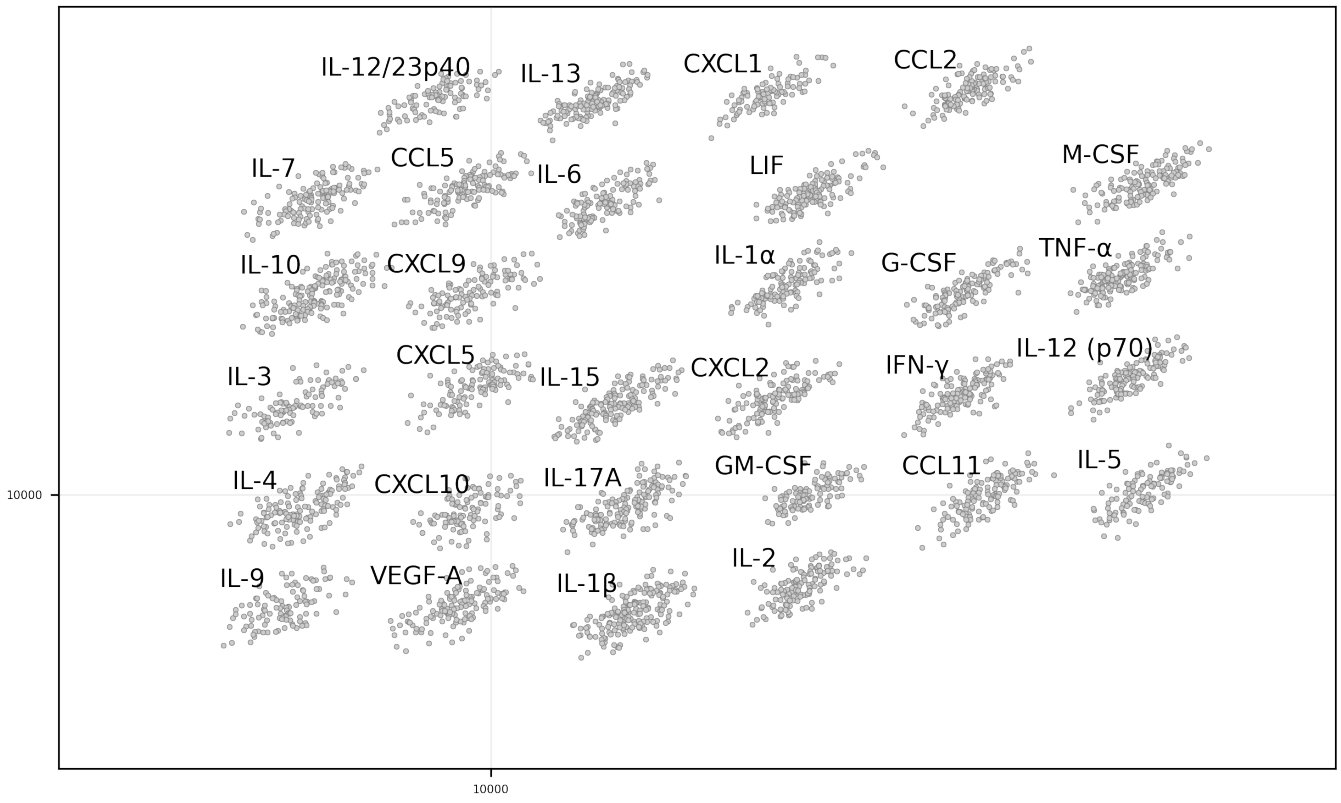
	Average recovery (%) in
Analyte	**Serum**
CXCL5 (LIX)	97 (dilution 1:32-1:256)
IL-4	90 (dilution 1:16-1:512)
M-CSF	99 (dilution 1:8-1:128)

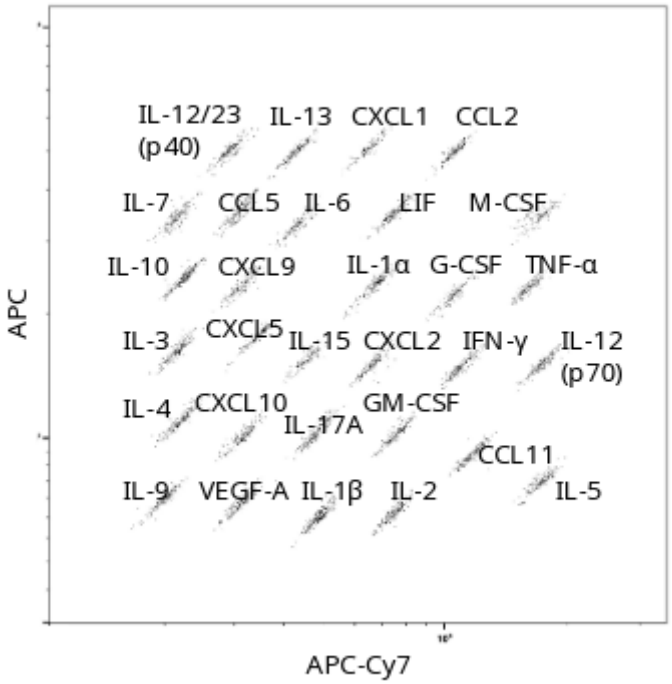
Linearity of dilution: Average % recovery (corresponding dilution range). Data was obtained from the first batch analysis.

Bead ID

Analyte	Bead ID
CCL2 (MCP-1)	B3
CCL5 (RANTES)	E4
CCL11 (Eotaxin)	B7
CXCL1 (KC/GRO-α)	C3
CXCL2 (MIP-2)	C6
CXCL5 (LIX)	E6
CXCL9 (MIG)	E5
G-CSF	B5
GM-CSF	C7
IFN-γ	B6
IL-1α	C5
IL-1β	D8
IL-2	C8
IL-3	F6
IL-4	F7

Analyte	Bead ID
IL-5	A7
IL-6	D4
IL-7	F4
IL-9	F8
IL-10	F5
IL-12 (p70)	A6
IL-12/-23 (p40)	E3
IL-13	D3
IL-15	D6
IL-17A	D7
IP-10 (CXCL10)	E7
LIF	C4
M-CSF	A4
TNF-α	A5
VEGF-A	E8




IL-12/23
(p40)
IL-13
CXCL1
CCL2
IL-7
CCL5
IL-6
LIF
M-CSF
IL-10
CXCL9
IL-1α
G-CSF
TNF-α
IL-3
CXCL5
IL-15
CXCL2
IFN-γ
IL-12
(p70)
IL-4
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IL-17A
CCL11
IL-9
VEGF-A
IL-1β
IL-2
IL-5
APC
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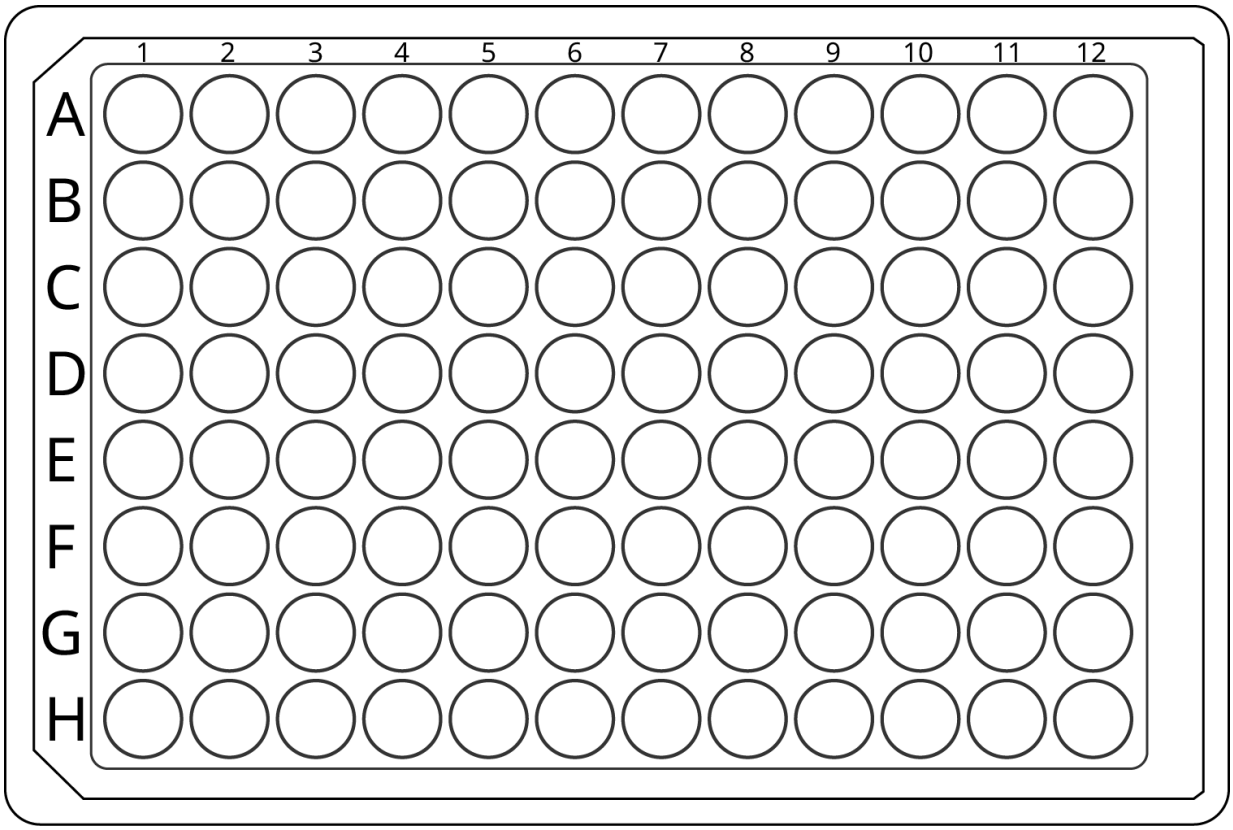

Note:
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