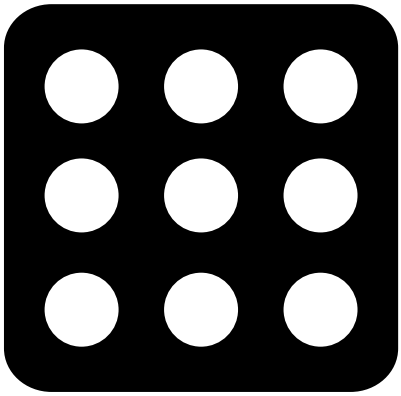
EYRAplex: Human Th1/Th2 (7-plex)

Product code: 5410-1EY-1



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Intended use

EYRAplex: Th1/Th2 (7-plex) is intended for the quantification of human IFN-γ, IL-2, IL-4, IL-5, IL-6, IL-12 (p70), and TNF-α in serum, plasma, and cell culture supernatants. The assay is designed for analysis using Mabtech EYRA™. 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: Human Th1/Th2 (7-plex)	-	6 ml
Detection mAb mix: Human Th1/Th2 (7-plex)	1:100	60 µl
Standard mix A: EYRAplex human	See Standard datasheet	2
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

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

Shipping and storage

- Shipped with cold packs.
- 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) available on our website.

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

The magnetic beads settle quickly. Vortex the Capture bead mix immediately before use and at regular intervals (at least every 30 seconds) while adding to the plate.

Standard mix

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

Assay diluent

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

Plate handling and adhesive plate cover

Do not write on the adhesive plate cover. Ensure that both the cover and plate (underside) are clean and free from reagent splashes and fingerprints. When transferring the plate to the EYRA reader, handle it carefully to avoid disrupting the settled beads. Remove the black 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 lid during incubations, placed over the adhesive cover, to protect from light exposure.

Plate and adhesive plate cover

Do not write on the adhesive plate cover and ensure that both the cover and plate are clean and free from reagent splashes.

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 standard and dilute to create the standard curve according to the 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 cells 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 to ensure optimal assay performance. Perform the dilutions in tubes or plates, and always add the Assay diluent 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, depending on expected analyte concentrations.

Detection mAb mix
Dilute the Detection mAb mix in Assay Diluent according to the contents table and filter through a 0.2 µm filter. 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 Preparation section.

1. **Capture beads**
   Vortex the Capture bead mixture 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 General guidelines and Preparations.

3. **Standards and samples**
   Add 50 µl per well of standard mix and samples diluted in Assay diluent. Also include wells with 50 µl of Assay Diluent without standard, which will serve as assay background controls (blank wells). Cover the plate to protect from light and incubate for 2 hours at room temperature, 800 RPM, on an orbital shaker.

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

5. **Detection mAb mix**
   Add 50 µl per well of the diluted and filtered Detection mAb mix. Cover the plate 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 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 per well.

10. **For EYRA analysis**: 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 lid and insert the plate into the EYRA instrument. The adhesive plate cover may remain in place during reading.
    Note: Alternatively, the plate may be stored overnight at +4-8 °C, protected from light. If stored, repeat the shaking (5 minutes at 800 RPM) and settling (20 minutes) steps before reading.

Performance

Precision

Analyte	Intra-assay CV(%)	Inter-assay CV(%)
IFN-γ	4	8
IL-2	3	7
IL-4	4	18
IL-5	3	9
IL-6	4	7
IL-12 (p70)	3	9
TNF-α	3	5

Accuracy

	Average Recovery (%) in			
Analyte	**Heparin plasma**	**Citrate plasma**	**EDTA plasma**	**Serum**
IFN-γ	92	78	65	80
IL-2	88	105	98	89
IL-4	109	139	133	*
IL-5	89	91	87	92
IL-6	81	100	92	91
IL-12 (p70)	62	99	96	*
TNF-α	88	97	112	68

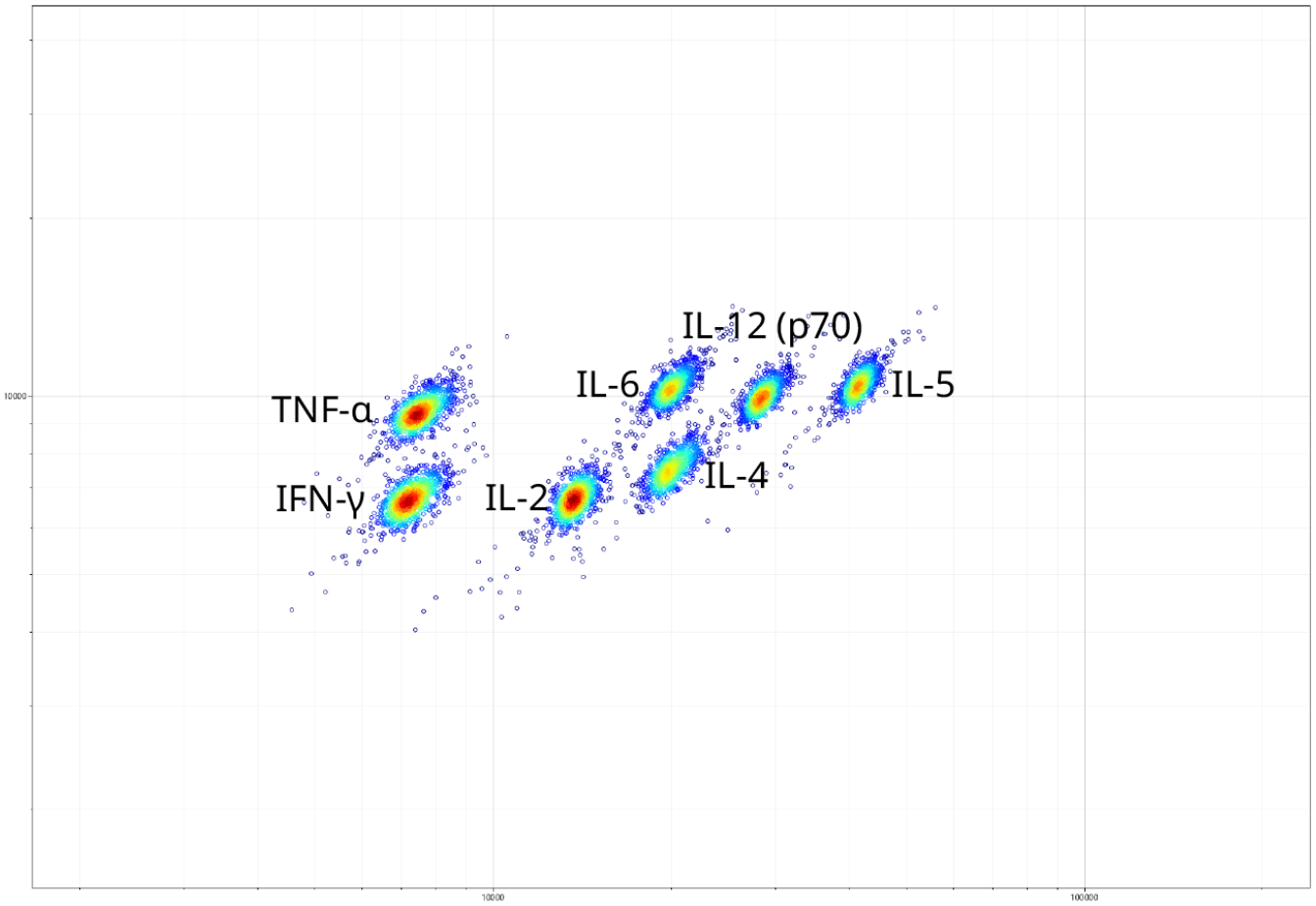
* Not recommended

Bead ID and gates

Bead ID

Analyte	Bead ID
IFN-γ	F8
IL-2	D8
IL-4	C8
IL-5	A7
IL-6	C7
IL-12 (p70)	B7
TNF-α	F7

Analytes and bead ID.



Note:
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Mabtech AB (Head Office)

Sweden
Tel: +46 8 716 27 00
mabtech@mabtech.com

Mabtech, Inc.

USA
Tel: +1 513 871-4500
mabtech.usa@mabtech.com

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