LUNARIS™ Protein Multiplexing Platform

LUNARIS™ Human 11-Plex Chemokine Kit

Analytes

CCL2	CCL20
CCL3	CXCL1
CCL4	CXCL10
CCL5	CXCL12
CCL11	IL-8
CCL19	

Handbook Version 06_2018

For the detection of multiple chemokines in human serum and cell culture supernatant

Cat. No.

1 x 32 wells	LHCK-10110S	1 x 96 wells	LHCK-20110S
3 x 32 wells	LHCK-10110F	4 x 96 wells	LHCK-20110F

This manual should be read in its entirety before using this product.

For Research Use Only. Not for use in diagnostic procedures.

AYOXXA — The translational proteomics company

LUNARIS™ is AYOXXA's innovative and patented beads-on-a-chip technology platform to measure picogram quantities of proteins in precious biological samples. It is a fully integrated multiplex protein analysis system consisting of a dedicated reader, integrated analysis software and an expanding menu of catalog kits. AYOXXA facilitates quantitative protein analysis — from basic research to the clinic.

AYOXXA sets standards in:

- Low-volume multiplex protein analysis
- Data quality and transparency
- Flexibility in format and content

For more information, visit www.ayoxxa.com.

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Kit contents and storage

LUNARIS™ Human 11-Plex Chemokine Kit					
Format	**96-well**		**384-well**		
Size **Catalog No.**	**1 × 32 wells** **LHCK-10110S**	**3 × 32 wells** **LHCK-10110F**	**1 × 96 wells** **LHCK-20110S**	**4 × 96 wells** **LHCK-20110F**	
Component					**Store at**
LUNARIS™ BioChip	1 × 32-well	3 × 32-well	1 × 96-well	4 × 96-well	4–8°C
Assay Diluent 5	10 mL	20 mL	10 mL	2 × 20 mL	4–8°C
Antibody Diluent	1.3 mL	1.3 mL	1.3 mL	5.2 mL	4–8°C
Wash Buffer 1 (10X)	20 mL	20 mL	20 mL	3 × 20 mL	4–8°C use within 1 week after dilution
Wash Buffer 2 (10X)	5 mL	5 mL	5 mL	5 mL	4–8°C use immediately after dilution
Detection Antibody (dAb) Mix (10X)	0.132 mL	0.132 mL	0.132 mL	4 × 0.132 mL	4–8°C use immediately after dilution
Streptavidin-Phycoerythrin (SA-PE) (10X)	0.132 mL	0.132 mL	0.132 mL	0.528 mL	4–8°C use immediately after dilution
Standard Mix 100X (shipped separately on dry ice)	1 vial	3 vials	1 vial	4 vials	–20°C
Adhesive foil strips	3 strips	9 strips	3 strips	12 strips	–

LUNARIS™ Human 11-Plex Chemokine Kit					
Format	**96-well**		**384-well**		
Size **Catalog No.**	**1 × 32 wells** **LHCK-10110S**	**3 × 32 wells** **LHCK-10110F**	**1 × 96 wells** **LHCK-20110S**	**4 × 96 wells** **LHCK-20110F**	
Component					**Store at**
Absorbent paper	4 sheets	12 sheets	4 sheets	16 sheets	–
Reagent reservoir	4 pcs	12 pcs	4 pcs	16 pcs	–
USB stick LUNARIS™ Decoding Files	1 stick	1 stick	1 stick	1 stick	–
Standard Value Card	1	1	1	1	–
Handbook	1	1	1	1	–

All components of the kit are stable if stored as indicated. Do not use them when expired. Reconstituted standard must be used immediately. Refer to table above for use instructions after dilution.

Intended use

LUNARIS™ products are purely research tools and may not be used for applied diagnostic purposes. This product is not a medical device and may not be used as such.

LUNARIS™ products are manufactured with scientific care and according to accepted scientific standards reflecting the state of the art and the legal regulations. However, AYOXXA will not guarantee to be liable concerning the sustainability of the products for the customer's individual use.

All of our sales, services and provisions of materials are subject to AYOXXA's general terms, which are available at www.ayoxxa.com/AGB/.

Safety information

When working with chemicals and hazardous biological materials, always wear suitable laboratory attire, disposable gloves and protective eyewear. Consult the appropriate safety data sheets (SDS), which can be requested via e-mail: sales@ayoxxa.com.

For safety information regarding necessary instruments, consult the relevant instrument user manual.

Discard all samples and assay waste according to your institution and local safety regulations.

Introduction

AYOXXA offers assays to customers who require robust and flexible multiplex protein testing solutions. Developed under rigorous quality control, AYOXXA products offer simple protocols and exceptional assay stability paired with outstanding reproducibility and lot-to-lot consistency.

The LUNARIS™ Human 11-Plex Chemokine Kit enables quantification of 11 biomarkers that are important in inflammation and immune system regulation.

Principle and procedure

The LUNARIS™ Human 11-Plex Chemokine Kit provides a flexible and robust method to determine the concentration of multiple protein targets in small sample volumes. The assay combines the robustness of classical ELISA with the performance of modern bead-based immunoassays delivered on a two-dimensional surface (LUNARIS™ BioChip). With ready-to-use chips and reagents, the LUNARIS™ protein multiplex platform affords the ease of use known from classical ELISA, but delivers quantification of multiple targets in a single sample.

Thousands of beads bearing highly specific capture antibodies against the target proteins of the assay are immobilized onto the LUNARIS™ BioChip in a distinct pattern. The patented AYOXXA Technology generates a unique “fingerprint” file for each LUNARIS™ BioChip that codes the exact location of every individual bead on the chip.

During the experimental procedure, the user simply loads samples onto the LUNARIS™ BioChip and target analytes bind to the beads. In subsequent steps, secondary antibodies and a fluorescent label are added. Finally, a dedicated imaging device (LUNARIS™ Reader™) is used to detect and record the resulting fluorescent signal and the intuitive LUNARIS™ Analysis Suite automatically processes the readout data to provide detailed information on the concentration of targeted proteins in each sample.

The LUNARIS™ protein multiplex platform includes assay panels in formats from 32- to 384-wells. These formats can be used with the unique engineering of the LUNARIS™ BaseFrame to enable flexible scalability and meet user-specific throughput needs.

How to assemble the LUNARIS™ BioChip and BaseFrame

The system consists of three components (from bottom to top): the LUNARIS™ BaseFrame, 1–4 BioChips and a lid. The BaseFrame enables assays in 96 or 384 MTP-formats by rotating the frame 180 degrees.

You can use 1–3 32-well (96) or 1–4 96-well (384) BioChips to accommodate the number of samples you wish to test. Each BioChip is earmarked in the upper left corner to indicate how it engages with the pins of the LUNARIS™ BaseFrame.

Position BioChips next to one another.

BioChips must be be **firmly locked** into the BaseFrame. A distinct ***click*** -sound confirms the optimal vertical alignment for automatic readout.

Fully assembled LUNARIS™ BaseFrame and BioChips.

Close the lid to store and transport the LUNARIS™ BaseFrame and BioChips.

Our video tutorials give practical advice on how to set up the LUNARIS™ system. Visit our website www.ayoxxa.com to find out more.

Limitations of the procedure

Complex biological samples and matrices exhibit natural variation that may impact results (matrix effects). Use the LUNARIS™ Human 11-Plex Chemokine Kit only for the recommended matrices: serum, cell culture supernatant.

Equipment and reagents supplied by the user

Ensure that all equipment and instruments have been serviced and calibrated according to the manufacturer's instructions.

- 5–50 µl multichannel pipet and corresponding pipet tips
- Single-channel pipets and corresponding pipet tips
- 1.5 ml, 2 ml and 5 ml reaction tubes (e.g., Eppendorf® Protein LoBind Tubes)
- 15 and 50 ml tubes (e.g., Falcon® Centrifuge Tubes, Corning®)
- Deionized or distilled water
- Plate centrifuge (e.g., Thermo Megafuge 16R with M-20 Microplate Swinging Bucket Rotor)
- LUNARIS™ Reader™ (LRS-001)
- LUNARIS™ Accessory Kit (Cat. No. LAK-001)

Additional recommended equipment and reagents

- Low protein binding 96-well or 384-well microplate, depending on kit size used (e.g., Eppendorf® Protein LoBind Polypropylene Microplates)
- Common table centrifuge (e.g., Centrifuge 5415 D, Eppendorf®)
- Common shaker and vortexer

Tips and best practices

- Allow enough time for sample preparation. Depending on the number of samples, preparing dilutions may take over 1 hour. Please plan accordingly.
- Prepare all vials for dilutions prior to thawing samples in order to minimize the time between thawing and transfer to the LUNARIS™ BioChip.
- Equilibrate all buffers and solutions to room temperature (18–23°C) before use.
- Check all buffers for precipitates. If precipitate has formed, warm the bottle to room temperature before diluting.
- The use of a microtiter plate and multichannel pipet to transfer samples, standards, and blanks onto the LUNARIS™ BioChip synchronizes exposure of the sample analytes to the beads on the BioChip and makes incubation time uniform across all samples.
- Protocol steps may be automated on a microplate washer (e.g., BioTek® MultiFlo FX Multi-Mode-Dispenser). Please contact your AYOXXA sales representative for support.
- Avoid using any aspiration tool that converts a vacuum source into a non-reproducible aspiration system.
- Precise and accurate pipetting is a crucial factor to ensure high assay performance. For best practices, see the User Guide "Impact of pipetting techniques on precision and accuracy" (Ewald, K. 2015. Eppendorf AG)
- If the protocol cannot be completed in one day, you can pause the assay at two different steps, each with slight procedure modifications:

  Step 21 19**:** dry the BioChip at room temperature for at least 1.5 hrs in a laminar flow cabinet or overnight without laminar flow. Prevent direct light exposure and use the LUNARIS™ Lid.

  Step 22 19**:** store the **dry** BioChip at 4–8°C overnight (e.g., in a petri dish sealed with parafilm), to perform imaging and analysis the following day. Protect the LUNARIS™ BioChip from light and prevent water condensation. Ensure that wells are completely dry before imaging.

- **Step 7** 16**:** the standard protocol is optimized for an incubation of 3 hrs at room temperature. Alternatively, you may incubate for 16–18 hrs at 4–8°C for more flexibility. In this case, a change in the Quantifiable Range will occur for some analytes. Please contact AXOXXA for more information.

Our video tutorials give practical advice on how to work with the LUNARIS™ system. Visit our website www.ayoxxa.com to find out more.

This handbook includes hyperlinks to related topics. The icons following these hyperlinks indicate the page number of the related content (e.g., Sample collection and handling 13).

Throughout the protocol, we provide tips to streamline the workflow and make it easier to follow. You will find the following symbols annotating protocol steps.

Important. The step or information provided is important and requires special attention.

Tips and Tricks. The information helps streamline or align workflow steps.

Incubation time. Indicates the time interval for an incubation step.

Preparation time. Lists the time required for a preparation step so it can be timed with other protocol steps.

Reagent, sample, standard preparation. Highlights information for the preparation of reagents, samples, or standards (e.g., reagent to prepare, page number, timing, etc.)

Video tutorial. A video is available to provide practical help. Please visit our website www.ayoxxa.com to find out more.

Dark incubation/storage. Designates steps (e.g., incubation, storage) that must be completed in a dark setting.

Pipetting. Indicates the volume to be pipetted to or from a vessel.

LUNARIS™ BioChip. Indicates the volume to be pipetted to or from the LUNARIS™ BioChip (e.g., volume transfer, wash steps, etc.)

Readout & analysis. Designates the point at which readout of the LUNARIS™ BioChip is performed. Consult the LUNARIS™ Analysis Suite.

Cold storage. Indicates cold storage and lists specific storage temperature.

Centrifugation. Indicates a procedure to concentrate liquid in the wells of a BioChip using a plate centrifuge.

Reverse centrifugation. Remove any residual liquid from the wells of a BioChip by centrifuging the assembly upside down in a plate centrifuge.

Protocol

Sample collection and handling

Serum

1. Collect whole blood and allow clotting at room temperature (18–23°C) for 30 minutes.
2. Centrifuge for 10 minutes at 2000 × g and 4°C. Transfer the serum (supernatant) to a fresh polypropylene tube.
3. Serum may be used immediately or stored at –80°C. Aliquot serum samples before freezing to avoid freeze-thaw cycles.

Cell culture supernatant

1. Centrifuge samples according to your standard protocol to pellet cells.
2. Supernatant may be used immediately or stored at –80°C. Aliquot supernatant samples before freezing to avoid freeze-thaw cycles.

Reagent and sample preparation

Depending on number of samples, the preparation and dilution procedure may take more than 1 hour. To avoid protein degradation, prepare all vials for the dilution prior to thawing samples. Prepare samples on ice and then bring to room temperature (18–23°C) before transferring to the LUNARIS™ BioChip. Prepare the Standard **after** the samples and at room temperature.

Sample preparation

The following are the recommended minimum dilution factors for each matrix or sample type. Depending on the known endogenous level of CCL5 in certain serum samples, higher dilutions might be required to measure within the quantifiable range.

Matrix/sample type	Assay Diluent	Dilution factor
Serum	Assay Diluent 5	2
Cell culture supernatant	Assay Diluent 5	2

Standard preparation

To prepare the Standard, follow the procedure described on the **Standard Value Card** provided with the kit.

Our video tutorials give practical advice on how to set up a multiplexing experiment. Visit our website www.ayoxxa.com to find out more.

Reagent preparation

Prepare Wash Buffer 2, Antibody Detection Solution and SA-PE Solution just before use.

Wash Buffers 1 and 2

Dilute 5 mL Wash Buffer 1 (10X) with 45 mL distilled or deionized water. Mix thoroughly. Repeat this step if more buffer is required.

Dilute 1.5 mL Wash Buffer 2 (10X) with 13.5 mL distilled or deinonized water (refer to step 19 of the procedure 16). Mix thoroughly.

Antibody Detection Solution (refer to step 8 of the procedure 16)

Dilute the desired volume of Detection Antibody Mix (10X) with the respective volume of Antibody Diluent according to the following table.

Format	Detection Antibody Mix (10X)	Antibody Diluent
1 × 32 wells	40 µL	360 µL
3 × 32 wells	120 µL	1080 µL
1 × 96 wells	120 µL	1080 µL
4 × 96 wells	480 µL	4320 µL

SA-PE Solution (refer to step 13 of the procedure 16)

Dilute the desired volume of SA-PE (10X) with the respective volume of Wash Buffer 1 according to the following table.

Format	SA-PE (10X)	Wash Buffer 1 (1X)
1 × 32 wells	40 µL	360 µL
3 × 32 wells	120 µL	1080 µL
1 × 96 wells	120 µL	1080 µL
4 × 96 wells	480 µL	4320 µL

Blanks

As the blank, use the Assay Diluent corresponding to the sample type to be tested (refer to Standard preparation 14).

Procedure

1. Prepare all sample dilutions as described in Reagents and sample preparation 13.

Dilutions 13

2. To prepare the Standard, follow the procedure described on the **Standard Value Card** provided with the kit.

Standard Value Card

3. Dispense diluted standard, diluted samples, and blanks into wells of a standard 96 or 384-well microplate.

   Recommendation: Prepare sufficient sample volume for duplicates or triplicates.

Volume depends on number of samples

4. Prewash the wells of the LUNARIS BioChip prior to assay.

   Add 40 µL Wash Buffer 1 to each well and incubate for 15 min. Aspirate Wash Buffer 1 using a multichannel pipet.

   For convenience, you can use the alternative washing procedure 22.

40 µL Wash Buffer 1 14

5. Remove residual fluid by reverse centrifugation 21.

   Place a sheet of absorbent paper between the LUNARIS™ BaseFrame and Lid. Position the assembly upside down into a suitable plate centrifuge and spin for 1 min at 700 × g. Proceed immediately to step 6.

1 min at 700 × g

6. Using a multichannel pipet, transfer 5 µL of Standards, blanks, and samples to the LUNARIS™ BioChip. Spin down liquid for 1 min at 700 × g to ensure that the BioChip surface is covered.

   Note: This step ensures a synchronized incubation start for all samples and standards.

5 µL Standard 14, blanks 15, samples 14

1 min at 700 × g

7. Seal the LUNARIS™ BioChip with an adhesive foil strip (provided) and incubate for 180 min at room temperature. Alternatively, you may incubate overnight at 4°C to increase sensitivity for some analytes (refer to Tips and best practices) 10.

180 min

8. Prepare the Detection Antibody Solution 10 min before the incubation of the LUNARIS™ BioChip ends.

10 min

Detection Antibody Solution 15

9. Carefully remove the adhesive foil from the LUNARIS™ BioChip and discard. Use a multichannel pipet to aspirate samples and wash each well with 40 µL Wash Buffer 1. Repeat the washing step three times.

   For convenience, you can use the alternative washing procedure 22.

3 × 40 µL Wash Buffer 1 14

10. Remove residual fluid by reverse centrifugation 21.

    Place a sheet of absorbent paper between the LUNARIS™ BaseFrame and Lid. Position the assembly upside down into a suitable plate centrifuge and spin for 1 min at 700 × g. Proceed immediately to step 11.

1 min at 700 × g

11. Pipet 10 µL Detection Antibody Solution into each well of the LUNARIS™ BioChip. Spin down liquid for 1 min at 700 × g to ensure that the BioChip surface is covered.

10 µL Detection Antibody Solution 15

1 min at 700 × g

12. Seal the LUNARIS™ BioChip with an adhesive foil strip (provided) and incubate for 60 min at 18–23°C.

60 min

18–23°C

13. Prepare the SA-PE Solution 10 min before the incubation of theLUNARIS™ BioChip ends. Protect from light.

10 min

SA-PE Solution 15

14. Carefully remove the adhesive foil from the LUNARIS™ BioChip and discard. Use a multichannel pipet to aspirate the Detection Antibody Solution and wash each well with 40 µL Wash Buffer 1. Repeat the washing step three times.

For convenience, you can use the alternative washing procedure 22.

3 × 40 µL Wash Buffer 1 14

15. Remove residual fluid by reverse centrifugation 21.

Place a sheet of absorbent paper between the LUNARIS™ BaseFrame and Lid. Position the assembly upside down into a suitable plate centrifuge and spin for 1 min at 700 × g. Proceed immediately to step 16.

1 min at 700 × g

16. Add 10 µL SA-PE Solution into each well of the LUNARIS™ BioChip using a multichannel pipet. Spin down liquid for 1 min at 700 × g to ensure that the BioChip surface is covered.

10 µL SA-PE Solution 15

1 min at 700 × g

17. Seal the LUNARIS™ BioChip with an adhesive foil strip (provided) and cover it with the Lid to protect the samples from light. Incubate for 30 min at 18–23°C.

Protect from light

30 min


18–23°C

18. Carefully remove the adhesive foil from the LUNARIS™ BioChip and discard. Use a multichannel pipet to aspirate the SA-PE Solution and wash each well with 40 µL Wash Buffer 1. Repeat the washing step five times.

For convenience, you can use the alternative washing procedure 22.


5 × 40 µL Wash Buffer 1 14

19. Wash each well with 40 µL Wash Buffer 2 using a multichannel pipet.


1 × 40 µL Wash Buffer 2 14

20. Remove residual fluid by reverse centrifugation 21.

Place a sheet of absorbent paper between the LUNARIS™ BaseFrame and Lid. Position the assembly upside down into a suitable plate centrifuge and spin for **2 min** at 700 × g.

2 min at 700 × g

The protocol can be paused overnight after step 21 or step 22. See Tips and best practices 10 for instructions.

21. Allow the LUNARIS™ BioChip to dry in a laminar flow cabinet for at least 1.5 hours. Without laminar flow, extend drying time for an additional hour to overnight. Prevent light exposure: switch off the cabinet light and use the Lid.

    Ensure that wells are completely dry before imaging.

Dark

≥1.5 h

22. Image the LUNARIS™ BioChip using the LUNARIS™ Reader™ (LRS-001) or a fluorescence microscope (Zeiss® Axio Imager.M2). Consult the LUNARIS™ Analysis Suite for readout settings.

    The dry LUNARIS™ BioChip can be stored overnight at 4–8°C.

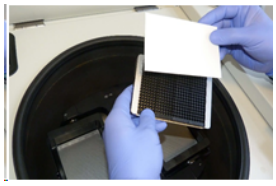

Readout and analysis 20

Readout and analysis

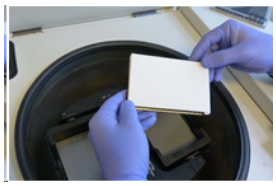
The presence of assay biomarkers in the samples, controls, and standards generates a fluorescent signal that is detected with the LUNARIS™ Reader™ (LRS-001). Quantification of the readout is performed entirely by the LUNARIS™ Analysis Suite included in the LUNARIS™ Accessory Kit. For details about readout analysis, consult the LUNARIS™ Analysis Suite User Manual.

Appendix

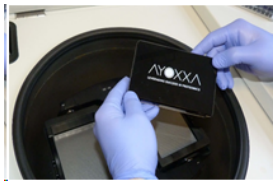
Reverse centrifugation



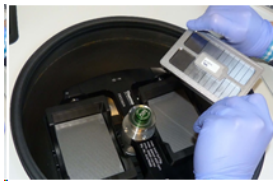
Place a sheet of absorbent paper (provided with the kit) on top of the assembled LUNARIS™ BioChip.



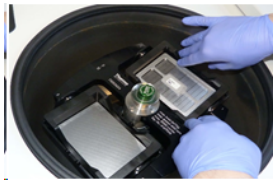
Align the absorbent paper with the edges of the BaseFrame.



Cover the BaseFrame with the Lid, ensuring it closes well.



Turn the assembled LUNARIS™ BaseFrame, BioChips, absorbent paper, and Lid **upside down**.



Position the assembled unit in a plate centrifuge.

Note: Always balance centrifuge rotors! For details, refer to your centrifuge manual.

Spin for 1 or 2 min at 700 × g maximum, and then proceed immediately with the next step of the protocol.

Our video tutorials give practical advice on how to remove liquid from wells of the LUNARIS™ BioChip efficiently. Visit our website www.ayoxxa.com to find out more.

Alternative washing procedure

The following instructions may be useful to simplify and expedite washing steps in the overall procedure.

Plate tapping:

Step 9: Add 40 µL of Wash Buffer 1 to each well containing 5 µL of standard, blank, control or sample. Then, discard the fluid in the wells by inverting and flicking the plate over a sink, and repeatedly tapping the plate on clean paper towels. Repeat the wash and discard steps the number of times indicated in the procedure.

Steps 14 and 18: Without adding Wash Buffer 1 first, discard the 10 µL of Antibody Detection Solution or SA-PE Solution by inverting and flicking the plate over a sink and repeatedly tapping the plate on clean paper towels. Then add 40 µL of Wash Buffer 1 to repeat the wash and discard steps the number of times indicated in the procedure.

Our video tutorials show you how to perform plate tapping. Visit our website www.ayoxxa.com to find out more.

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