MANUAL

RayBio® Ultrasensitive Antibody Pair

Catalog #: RMH-CUST
Size: 96 tests

ISO 13485:2016

Summary

The RayBio® Ultrasensitive Antibody Pair kit supplies essential components for the application of Single Molecule Array (Simoa) for quantitative detection of target proteins in a wide range of biological samples such as cell culture media, human serum, and plasma. This kit is for research use only and not for clinical diagnostics.

Storage

The entire kit may be stored at 4 °C upon arrival and used in 2 weeks. For long-term storage, keep the conjugated beads and detector antibody at 4 °C and the calibrator below -70 °C for up to 6 months.

Reagents

Component	Size	Description
Conjugated Beads	1 vial	Capture beads conjugated with target protein in Homebrew Bead Diluent buffer enough for 96 tests. Dilute as instructed before use.
Calibrator	2 vials	Lyophilized standard protein.
Detector Antibody	1 vial	Biotinylated detector antibody. Dilute as instructed before use.

Additional Equipment and Materials Required

1. Simoa HD-X Analyzer®
2. Simoa HD-1/HD-X System Wash Buffer 1 and Wash Buffer 2
3. Simoa HD-1/HD-X Sealing Oil
4. Homebrew Detector/Sample Diluent
5. Homebrew Bead Diluent Buffer
6. Simoa cuvettes, discs, pipette tips, 96-well microplates, and Pierce XP-100 Plate Seals
7. Quanterix SBG Concentrate and SBG Diluent
8. Quanterix RGP (1 bottle per 48 tests)
9. Reagent bottles for Simoa HD-1/HD-X Analyzer and homebrew barcoded labels

Reagent Preparation

Follow the instructions to prepare material for 96 tests. If less than 96 tests are planned, calculate the volume needed for each component. Only use buffer solutions from Quanterix or supplied in this kit for the best results.

1. Conjugated beads: Vortex the tube for 30 seconds and briefly centrifuge the tube to collect beads from the cap. Pipette all beads into a conical bottom Simoa reagent bottle and dilute the beads with 4428 µL Homebrew Bead Diluent Buffer. Apply an appropriate bar-coded label to the bottle and mark it as Bead Reagent. Store diluted beads at 4 °C, avoiding light. Vortex the bottle for at least 30 seconds before installing into Simoa HD-X Analyzer.

2. Calibrator: Reconstitute lyophilized antigen in the Homebrew Detector/Sample Diluent.

3. Detector Antibody: Briefly spin down the vial, add desired amount of Homebrew Detector/Sample Diluent and gently mix the diluted antibody by pipetting. Transfer the diluted detector antibody into a conical bottom Simoa reagent bottle. Apply an appropriate barcoded label to the bottle and mark it as Detector Reagent. Mix well before use. Store diluted antibody at 4 °C.

4. RGP (NOT provided in this kit): Place RGP bottles (each bottle is sufficient for 48 tests on the HD-X platform) in a heated shaker for at least 30 minutes at 800 RPM at 30 °C. For best performance, do not store and reuse opened RGP bottles. Refer to the manufacturer protocol for details.

5. SBG solution (NOT provided in this kit): Concentrated SBG solution and SBG diluent are obtained from Quanterix. The optimal concentration should be tested and determined based on the sample type and experimental settings. Apply an appropriate barcoded label to the bottle and mark it as SBG Reagent. Mix well before use.

Simoa Assay Description

Recommended assay format	2-step digital immunoassay
Recommended curve fitting	4-parameter logistic (4PL), $1/y^2$ weighting
Total test per kit	96
Typical run setup	8 points of calibrators in triplicate 36 samples in duplicate
Minimum calibrator volume	130 µL for 1 replicate per well; 230 µL for duplicate per well
Minimum sample volume	130 µL for 1 replicate per well; 230 µL for duplicate per well
Recommended sample dilution	Human serum and plasma samples are recommended to dilute 4x

Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.

1. Plasma samples:

Collect blood with an anticoagulant such as citrate or EDTA and mix by inversion (*Note:* Chelating anticoagulants cannot be used). Centrifuge the blood at 1000 x g at 4 °C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80 °C for storage. Plasma is recommended to dilute 4x to use in the assay.

2. Serum samples:

Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80 °C for storage. Serum is recommended to dilute at least 4x to use in the assay.

Assay Procedure

A freshly prepared standard curve should be used each time the assay is performed.

Step 1: Reagent preparation. Remove Conjugated Beads and Detector Antibody from 4 °C and keep the vials on ice until use. Remove Calibrator from 4 °C/-80 °C and thaw it at room temperature. Prepare working solutions, including Conjugated beads, Detector antibody, Calibrator, RGP and SBG solution, according to the **Reagent Preparation** section above.

Step 2: Create a homebrew assay definition. Go to the Custom Assay tab, add a new homebrew assay or modify an existing homebrew assay according to the **Simoa assay description** section above. Refer to the Homebrew Assay Development Guide for additional information on setting up the assay definition.

Step 3: Sample preparation. Plasma and serum samples require at least 4x dilution for this assay. At-bench dilutions are recommended. To ensure accurate results, avoid debris in samples while making dilutions. If choosing on-board dilutions by the HD-X instrument, refer to the Homebrew Assay Development Guide for additional information on sample preparation and loading.

Step 4: Plate calibrators and samples. For a typical assay setup, all calibrators are run in triplicates and all samples are run in duplicates. Load ≥ 130 µL of samples or calibrators per well on a Quanterix 96-well microplate for 1 replicate. For 2 replicates in a well, load ≥ 230 µL of samples or calibrators. Avoid bubbles when loading and do not exceed 300 µL volume in any wells. Carefully apply a Pierce XP-100 on top of the loaded plate with circles centered over the wells. Gently press the seal on the plate.

Step 5: Install reagents on HD-X. The capture beads need to be vortexed for at least 30 seconds immediately before use. Load Bead Reagent, Detector Reagent and SBG Reagent into a reagent rack. Register the reagents by manually or automatically scanning the bar codes. Update the volume information of every reagent at the first use. Sufficient RGP bottles should be scanned and loaded into the RGP rack.

Step 6: Assay setup. Create or revise the Homebrew assay definition following instructions in the **Simoa assay description** section. Select the suitable assay definition for calibrators and samples with the Neat protocol (recommended). If the samples will be diluted by the HD-X instrument, a different assay definition and a bottle of Sample diluent are needed. For more details about on-board dilution, refer to the Homebrew Assay Development Guide. Comprehensive results and statistics are included in run history and reports. Maintain the HD-X instrument as required at the end of the experiment.

Determination of target concentration in human samples

Obtain the run history and report from HD-X. See HD-X Data Analysis Guide for details.

This product is for research use only.