Quanterix

Simoa™ Qualification Test

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

Read this package insert prior to use. Package insert instructions must be carefully followed. Reliability of assay results cannot be assured if there are deviations from the instructions in this package insert.

NAME

Simoa™ Qualification Test

INTENDED USE

The Simoa Qualification Test (SQT) is a digital immunoassay used to assess the precision of the HD-1 Analyzer. The SQT may be used upon completion of the Monthly Maintenance Procedure (refer to *Simoa™ HD-1 Analyzer Monthly Maintenance Instructions*). The SQT may also be used when precision of an assay does not meet expected results. For optimal system performance, it is critical to follow all routine maintenance procedures defined in the *Simoa HD-1 Analyzer User Guide*.

PRINCIPLES OF THE PROCEDURE

The Simoa Qualification Test (SQT) is a 3-step digital immunoassay which uses immunoassay reagents to qualify the precision of Average Enzyme per Bead (AEB) of a control in the digital range of the assay and control in the analog range of the assay using the Simoa HD-1 Analyzer and Single Molecule Array (Simoa) technology. The test also assesses the performance of the Simoa HD-1 Analyzer by measuring the average bead fill as well as bead fill CV's.

In the first step, coated paramagnetic capture beads are combined with sample. Molecules present in the sample are captured by the coated capture beads. After washing, biotinylated detector antibodies are mixed with the capture beads. The detector antibodies bind to the captured molecules. Following a second wash, a conjugate of streptavidin-ß-galactosidase (SBG) is mixed with the capture beads. SBG binds to the biotinylated detector antibodies, resulting in enzyme labeling of captured analyte. Following a third wash, the capture beads are resuspended in a resorufin ß-D-galactopyranoside (RGP) substrate solution and transferred to the Simoa Disc. Individual capture beads are then sealed within microwells in the array. If analyte has been captured and labeled, the ß-galactosidase hydrolyzes the RGP substrate into a fluorescent product that provides the signal for measurement. A single labeled molecule results in sufficient fluorescent signal in 30 seconds to be detected and counted by the Simoa optical system.

For additional information on system and assay technology, refer to the *Simoa HD-1 Analyzer User Guide*. For information on how to generate SQT reports automatically, please refer to the *Customer Support Tool Users Guide*. Both documents are available on the Customer Portal.

REAGENTS

Reagent Kit

SQT kit (203)

Capture Bead Reagent	1 bottle	Capture antibody (mouse monoclonal) coated capture beads in Tris buffer with protein stabilizers (bovine). Preservative: ProClin 300.
Detector Reagent	1 bottle	Biotinylated detector antibody (mouse monoclonal) in phosphate buffer with protein stabilizers (bovine). Preservative: ProClin 300.
SBG Reagent	1 bottle	Conjugate of streptavidin-ß-galactosidase (SBG) in phosphate buffer with protein stabilizers (bovine). Preservative: ProClin 300.
RGP Reagent	1 bottle	Resorufin ß-D-galactopyranoside (RGP) in phosphate buffer with a surfactant.
SQT Control 1	2 vials	Antigen in phosphate buffer with protein stabilizers (bovine). Preservative: ProClin 300.
SQT Control 2	2 vials	Antigen in phosphate buffer with protein stabilizers (bovine). Preservative: ProClin 300.

Other Reagents

System Wash Buffer 1 (ordered separately)

- Phosphate buffer with surfactant. Preservative: ProClin 300.

System Wash Buffer 2 (ordered separately)

- Phosphate buffer. Preservative: ProClin 300.

Sealing Oil (ordered separately)

- Synthetic fluorinated polymer.

WARNINGS AND PRECAUTIONS

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Safety Precautions

- The SQT reagents contain methylisothiazolones, which are components of ProClin and are classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.

R43 May cause sensitization by skin contact.

S24 Avoid contact with skin.

S35 This material and its container must be disposed of in a safe way.

S37 Wear suitable gloves.

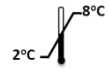
S46 If swallowed, seek medical advice immediately and show this container or label.

- For a detailed discussion of safety precautions during instrument operation, refer to the *Simoa HD-1 Analyzer User Guide*.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not pool reagents within a kit or between reagent kits.
- Do not attempt to reuse tips, cuvettes or Simoa Discs, as this will cause significant data quality deterioration.
- Prior to loading the SQT reagents on the Simoa HD-1 Analyzer, the Bead Reagent bottle must be mixed to resuspend capture beads that have settled during shipment. For capture bead–mixing instructions, refer to the Procedure section of this package insert.
- For a detailed discussion of handling precautions during instrument operation, refer to the *Simoa HD-1 Analyzer User Guide.*

Storage Instructions

- 

SQT Reagents must be stored at 2–8°C in an upright position.
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SQT Controls must be stored at -20°C.

PROCEDURE

Materials Provided

SQT Reagent Kit

Materials Required But Not Provided

- Simoa HD-1 Analyzer
- Simoa HD-1 System Wash Buffer 1
- Simoa HD-1 System Wash Buffer 2
- Simoa HD-1 Sealing Oil
- Simoa cuvettes
- Simoa disposable pipettor tips
- Simoa Discs
- For information on materials required for maintenance procedures, refer to the *Simoa HD-1 User Guide*.

Instrument Procedure

- The SQT assay definition must be installed on the Simoa HD-1 Analyzer prior to performing the assay. The assay definition is available to download from the Quanterix Customer Portal. For best results, please be sure to check the Customer Portal prior to running this kit for the latest SQT assay definition, as these may change without notice. For more information on obtaining and installing the assay definition, refer to the *Simoa HD-1 Analyzer User Guide*.

Assay Procedure

- Remove reagents and control tubes from storage and allow them to come to room temperature for a minimum of 1 hour (up to a maximum of 5 h) before loading onto the HD-1 Analyzer. Thoroughly mix the controls by 20x gentle inversion.

  Note: Incompletely acclimated and mixed controls may give inconsistent results.
- Before loading the SQT reagents on the Simoa HD-1 Analyzer, the Bead Reagent bottle must be mixed to resuspend capture beads that have settled during shipping and storage. To resuspend the beads, vortex for a **minimum** of 30 seconds. (Note: The bead reagent is formulated with an antifoam agent, but vortexing can still create foaming. If the foam does not dissipate within a few minutes, remove excess foam with a pipette prior to loading capture beads onto the Simoa HD-1 Analyzer.)
- Before loading a new bottle of RGP Reagent on the Simoa HD-1 Analyzer for the first time, mix the reagent by inversion 10 times to ensure homogeneity.
- Set up the assay run on the instrument (see the *Simoa HD-1 Analyzer User Guide*).
  - Load the SQT reagents (Bead Reagent, Detector Reagent and SBG Reagent into the reagent bay.
  - Prior to loading controls, go to the Setup Run screen and scan the barcode and select 24 replicates for each control tube (2 tubes supplied for each control); then invert each control tube 3 times to mix and place in the sample bay in rack mode on the HD-1 Analyzer.
  - Load RGP into the sample bay. (Note: Only one bottle of RGP is needed per 96-sample run.)
- Replenish consumables and system resources as needed prior to initiating the run, as described in the *Simoa HD-1 Analyzer User Guide*.
- Initiate the run.

During the run the Simoa HD-1 Analyzer performs the following functions:

- Primes washers and sealing oil tubing and washes the fixed tip pipettor.
- Adds cuvettes to the incubation ring.
- Adds sample to the cuvette and shakes the cuvette to mix beads and sample.
- Incubates the capture bead–sample mixture.
- Moves the cuvette to a wash station and washes capture beads with System Wash Buffer 1.
- Removes System Wash Buffer 1 from the cuvette and adds Detector Reagent, followed by shaking and incubation.
- Moves the cuvette to a wash station and washes capture beads with System Wash Buffer 1.
- Removes System Wash Buffer 1 from the cuvette and adds SBG Reagent, followed by shaking and incubation.
- Moves the cuvette to a wash station and washes capture beads with System Wash Buffer 1. The final wash uses System Wash Buffer 2 to remove surfactant.
- Removes System Wash Buffer 2 from the cuvette and adds RGP Reagent.
- Transfers the capture bead mixture to the inlet port of a Simoa Disc.
- Applies negative pressure to the flow cell from a ventilation port in the disc, drawing the bead mixture across the readout array. Beads settle into wells of the array.
- Indexes the Simoa Disc to the sealing oil station and pipettes oil into the inlet port of the disc. The oil seals the wells of the array.
- Indexes the Simoa Disc to the imaging station and interrogates the array for the presence of fluorescence signal growth in each well of the array.
- Analyzes the array images to determine the average enzymes/bead (AEB).

For information on setting up assay runs, data analysis, and general operating procedures, refer to the *Simoa HD-1 Analyzer User Guide*.

For optimal performance, it is critical to follow the routine maintenance procedures defined in the *Simoa HD-1 Analyzer User Guide*.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the *Simoa HD-1 Analyzer User Guide*.

ACCEPTANCE CRITERIA

It is recommended to run the SQT kit after performing Monthly Maintenance or any time the assessment of precision and performance of the HD-1 Analyzer is desired. The precision and performance results will provide the user with guidance as to the data quality to expect when running their own assays. In order to assess system precision, the user can automatically generate an SQT Report using the Customer Support Tool. The acceptance criteria for the SQT test are:

ACCEPTANCE CRITERIA	
Control 1 Tube/Disc 1 AEB CV <	10%
Control 1 Tube/Disc 2 AEB CV <	10%
Control 2 Tube/Disc 1 AEB CV <	10%
Control 2 Tube/Disc 2 AEB CV <	10%
# Outliers Removed ≤	2
Combined # Outliers & Image Failures ≤	3
Avg % Bead Fill >	12.50
% Bead Fill CV <	20%

If the results for each of these 8 criteria are within specifications, the report will display a PASS. If the results are outside of the specification, the report with display a FAIL. If the results show FAIL, the user may contact Quanterix Service at service@quanterix.com for further troubleshooting support.

LIMITATIONS OF THE PROCEDURE

For research use only.

BIBLIOGRAPHY

1. Rissin DM, Kan CW, Campbell TG, et al. Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nat Biotech 2010; 28:595–99.

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