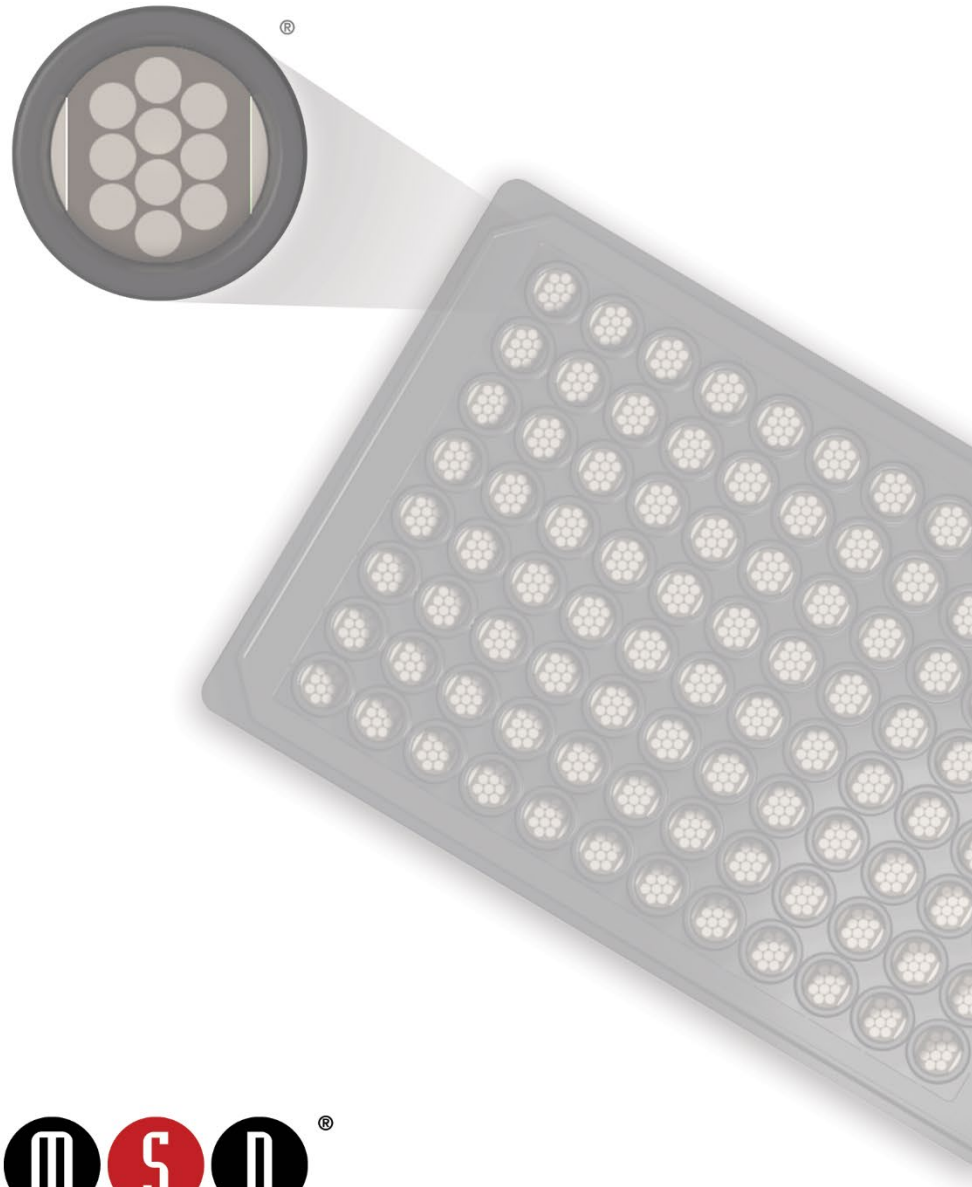
# MSD® MULTI-SPOT Assay System

## T1D Autoantibody Panel 1 (human) Kit

GADA, IA-2A, ZnT8A, IAA

V-PLEX®



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# MSD Autoantibody Assay

## GADA, IA-2A, ZnT8A, IAA

**For use with human serum and plasma samples.**

*This package insert should be read in its entirety before using this product.*

**FOR RESEARCH USE ONLY.**

**NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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# Contact Information

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# Introduction

This product insert describes the V-PLEX® Type 1 Diabetes Autoantibody (T1D) Panel 1 (human) Kit, lists the components, and provides instructions for use.

## Principle of the Bridging Assay

The V-PLEX T1D Autoantibody Panel 1 (human) Kit uses a bridging serology assay to measure levels of antibodies against endogenous antigens. The workflow consists of the following steps (see also Figure 1):

1. The antibody bridges a biotinylated antigen to a SULFO-TAG™ labeled antigen.
2. The bridged complex binds to its predetermined spot on the MSD plate.
3. The MSD instrument applies a voltage to the plate electrodes and measures the emitted light from the electrochemiluminescent SULFO-TAG label. The emitted light is proportional to the amount of analyte present in the sample.

## Product Introduction

Autoantibodies — immune proteins directed against the body's own tissues — are defining features of autoimmune diseases, including Type 1 Diabetes (T1D), rheumatoid arthritis, and Crohn's disease. In immune-mediated T1D, autoantibodies targeting insulin (IA), glutamic acid decarboxylase 65 (GAD65), zinc transporter 8 (ZnT8), and insulinoma-associated protein 2 (IA-2) are well-established biomarkers with proven predictive value, frequently detectable years before clinical disease onset.[2-4] The V-PLEX T1D Autoantibody Panel 1 (human) Kit enables simultaneous, quantitative detection of all four autoantibodies in serum or plasma — delivering a comprehensive humoral autoimmunity profile from a single well. This multiplexed approach conserves limited sample volumes, simplifies workflows, and supports high-throughput screening, making it equally suited for mechanistic research and large-scale epidemiological studies. Powered by non-radioactive electrochemiluminescence (ECL) detection, the kit removes the barriers associated with radioimmunoassay-based methods, broadening accessibility across diverse laboratory settings.

Biotinylated antigens locate to well-defined spots in each well, as shown in Figure 2. Antibodies bridge SULFO-TAG labeled antigens to the capture antigen, completing the assay.

1. GADA
2. –
3. IA-2A
4. –
5. –
6. –
7. –
8. ZnT8A
9. –
10. IAA

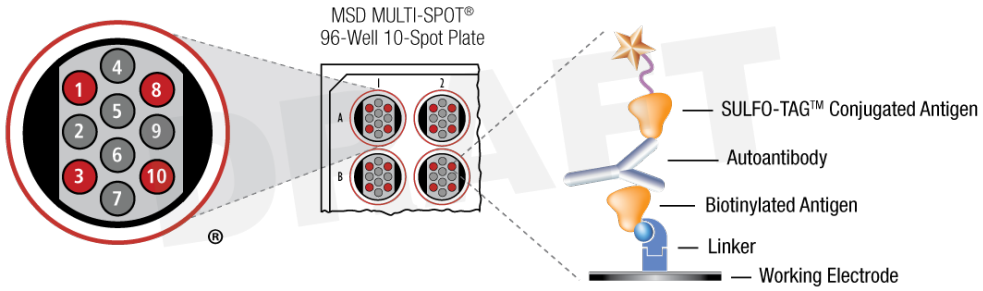


*Figure 1. Multiplex plate spot diagram showing the placement of capture antigens. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.*

# Kit Components

V-PLEX T1D Autoantibody Panel 1 (human) Kits include two positive controls. See below for details.

## Reagents Supplied with the Kit

*Table 1. Reagents that are supplied with V-PLEX T1D Autoantibody Panel 1 (human) Kit*

| Reagent | Storage | Catalog # | Size | Quantity Supplied | | | Description |
|---|---|---|---|---|---|---|---|
| | | | | 1-Plate Kit | 5-Plate Kit | 25-Plate Kit | |
| T1D Autoantibody Panel 1 (hu) 96-Well 10-Spot 4-Assay QuickPlex Ultra™ Plate | 2–8 °C | N0B757A-1 | 1 plate | 1 plate | 5 plates | 25 plates | 96-well plate, foil sealed, with desiccant |
| T1D Autoantibody Panel 1 (hu) 96-Well 10-Spot 4-Assay SECTOR™ Plate | 2–8 °C | N05757A-1 | 1 plate | 1 plate | 5 plates | 25 plates | 96-well plate, foil sealed, with desiccant |
| T1D Autoantibody Panel 1 (human) Calibrator Blend | ≤-70 °C | C0757-2 | 1 vial | 1 vial | 1 vial | 5 vials | Blend of four recombinant antibodies |
| Biotin Human GAD2/GAD-65 | ≤-70 °C | B2757-3 | 5 plate | 1 vial | 1 vial | 5 vials | Biotinylated and linker-coupled antigens |
| Biotin Human IA-2/R-PTP-N | | | | | | | |
| Biotin Human ZnT8 | | | | | | | |
| Biotin Human Insulin | | | | | | | |
| SULFO-TAG Human GAD2/GAD-65 | | | | | | | SULFO-TAG conjugated antigens |
| SULFO-TAG Human IA-2/R-PTP-N | | | | | | | |
| SULFO-TAG Human ZnT8 | | | | | | | |
| SULFO-TAG Human Insulin | | | | | | | |
| Diluent 100 | 2–8 °C | R50AA-4 | 50 mL | 1 bottle | 1 bottle | 5 bottles | Diluent for antigen dilution |
| Diluent 6 | ≤-10 °C | R53BB-4 | 8 mL | 1 bottle | 1 bottle | 5 bottles | Diluent for calibrator and sample dilution |
| MSD Wash Buffer (20X) | RT | R61AA-1 | 100 mL | 1 bottle | 1 bottle | 5 bottles | 20-fold concentrated phosphate buffered solution with surfactant |
| T1D Autoantibody Panel 1 (human) Control 1 | ≤-70 °C | C5757-1 | 1 vial | 1 vial | 1 vial | 5 vials | Multi-analyte controls in a non-human matrix, spiked with recombinant antibodies. A lot-specific COA is available. |
| T1D Autoantibody Panel 1 (human) Control 2 | ≤-70 °C | C5757-2 | 1 vial | 1 vial | 1 vial | 5 vials | |
| MSD GOLD™ Read Buffer B | RT | R60AM-1 | 18 mL | 1 bottle | — | — | Buffer to catalyze the electro-chemiluminescence reaction |
| | | R60AM-2 | 90 mL | — | 1 bottle | 5 bottles | |
| Adhesive Plate Seals | RT | – | – | 3 | 15 | 75 | Adhesive seals for sealing plates during incubations |

RT = room temperature
Dash (—) = not applicable

For more information on how the calibrators and antigens were produced, see Assay Components section below.

## Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing dilutions
- ❑ 96-well polypropylene U-bottom plate for preparing the assay mixture
- ❑ Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Microtiter plate shaker (rotary) capable of shaking at 500–1,000 rpm
- ❑ MSD Wash Buffer catalog no. R61AA-1
- ❑ Deionized water
- ❑ Vortex mixer

## Optional Materials and Equipment

- ❑ Centrifuge for sample preparation

## Catalog Numbers

MSD offers V-PLEX Assays designed for use on specific instrument platforms.

*Table 2. Catalog numbers associated with the V-PLEX T1D Autoantibody Panel 1 (human) Kit*

| Kit Name | SECTOR Plate | | | QuickPlex Ultra Plate | | |
|---|---|---|---|---|---|---|
| | 1-Plate Kit | 5-Plate Kit | 25-Plate Kit | 1-Plate Kit | 5-Plate Kit | 25-Plate Kit |
| V-PLEX T1D Autoantibody Panel 1 (human) Kit | K15757D-1 | K15757D-2 | K15757D-4 | K15757D-21 | K15757D-22 | K15757D-24 |

*Table 3. Instrument compatbiility for plate type*

| Plate Type | Instrument Compatibility |
|---|---|
| SECTOR Plate | MESO® SECTOR S 600, MESO SECTOR® S 600MM, MESO QuickPlex® SQ 120, MESO QuickPlex SQ 120MM |
| QuickPlex Ultra Plate | MESO QuickPlex Q 60MM |

**i** Ensure the plate type is compatible with your MSD instrument.

## Instrument Read Time

*Table 4. Instrument compatibility and read times*

| Instrument | 96-well SECTOR Plate | Plate Read Time | 96-well QuickPlex Ultra Plate | Plate Read Time |
|---|---|---|---|---|
| MESO QuickPlex SQ 120 | Y | 90 sec | N/A | N/A |
| MESO QuickPlex SQ 120MM | Y | 90 sec | N/A | N/A |
| MESO SECTOR S 600 | Y | 70 sec | N/A | N/A |
| MESO SECTOR S 600MM | Y | 70 sec | N/A | N/A |
| MESO QuickPlex Q 60MM | N/A | N/A | Y | 83 sec |

# Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Additional product-specific safety information is available in the applicable safety data sheet(s) (SDS), which can be obtained from MSD Customer Service or at *www.mesoscale.com*®.

# Best Practices

Read this product insert before use, and follow the best practices:

## Reagent Preparation

| | |
|---|---|
| **Do Not Mix Lots** | Do not mix components between boxes of multiplex V-PLEX panels. Lot information is provided in the lot-specific COA. |
| **Thaw Reagents** | Bring frozen diluents to room temperature in a 22–25 °C water bath. Thaw other reagents on wet ice and use them immediately. Mix well before use. Bring plates to room temperature before opening the packet. |

## Reagent Handling

| | |
|---|---|
| **Avoid Bubbles During Pipetting** | Avoid bubbles at each stage of reagent addition because they can lead to variable results. This is especially important when adding read buffer (just prior to reading the plate). |
| **Prepare in Polypropylene Microcentrifuge Tubes** | Prepare standards and samples in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution, and mix by vortexing after each dilution. |
| **Avoid Prolonged Light Exposure** | Avoid prolonged exposure of the detection reagent (stock or diluted) to light. During the plate incubation steps, plates do not need to be shielded from light (except for direct sunlight). |

## Plate Handling

| | |
|---|---|
| **Plate Shaking Guidelines** | Plate shaking should be vigorous, with a rotary motion between 500 and 1,000 rpm for 96-well plates. Keep the shaking speed and shaker model consistent for long-term studies. Binding reactions may reach equilibrium sooner if shaken in the middle of this range (~700 rpm) or above. |
| **Washing Fluid Removal** | Tap the plate on a paper towel after washing to ensure the removal of residual fluid. |
| **Some Assays Temperature Sensitive** | V-PLEX assays were characterized at 20–26 °C. Assays run above or below that range may be negatively affected. |

## Plate Reading

| | |
|---|---|
| **Remove Plate Seal** | Remove the plate seal before reading the plate in the instrument. |
| **Do Not Shake Plate** | Do not shake the plate after adding read buffer. |
| **Room Temperature Read Buffer** | Read buffer should be at room temperature (20-26 ºC) when adding it to the plate. |
| **Timing of Plate Reads** | Keep time intervals consistent between the addition of read buffer and reading the plate to improve interplate precision. Prepare an MSD instrument before adding read buffer. |
| **Results Above Curve** | If the sample results are above the top of the calibration curve, dilute the samples and repeat the assay. |

# Reagent Preparation and Protocol

Bring frozen diluents to room temperature in a 22–25 °C water bath before use. Thaw other reagents on wet ice and use them immediately.

## Prepare Wash Buffer

MSD provides 100 mL of Wash Buffer as a 20X stock solution. Dilute the stock solution to 1X before use.
For one plate, combine:

- ❑ 15 mL of MSD Wash Buffer (20X)
- ❑ 285 mL of deionized water

## Prepare Calibrator Dilutions

MSD supplies a multi-analyte calibrator blend that is provided at 5X the working concentration.
To prepare 7 calibrator solutions plus a zero calibrator for duplicate replicates (Figure 2):

1) Prepare the most concentrated calibrator (Calibrator 1) by transferring 25 µL of the 5X stock calibrator to 100 µL of Diluent 6. Mix by vortexing briefly.
2) Prepare the next calibrator by transferring 50 µL of Calibrator 1 to 100 µL of DIluent 6. Mix well by vortexing. Repeat 3-fold serial dilutions 5 additional times to generate 7 calibrators.
3) Use Diluent 6 as the zero calibrator.

Note: Thawed calibrator is stable for one day at 2–8 °C. It may also be stored frozen at ≤-70 °C and is stable through three freeze-thaw cycles. For the lot-specific concentration of each calibrator in the blend, refer to the COA supplied with the kit. You can also find a copy of the COA at www.mesoscale.com.

*Table 5. Serial dilution to generate the standard curve for 1 plate*

| Calibrator Standard No. | Tube No. | Source of Calibrator | Volume of Reconstituted Calibrator (µL) | Diluent 6 (µL) | Total volume (µL) |
|---|---|---|---|---|---|
| 1 | 1 | 5x stock calibrator (top of curve) | 25 | 100 | 125 |
| 2 | 2 | From tube 1 | 50 | 100 | 150 |
| 3 | 3 | From tube 2 | 50 | 100 | 150 |
| 4 | 4 | From tube 3 | 50 | 100 | 150 |
| 5 | 5 | From tube 4 | 50 | 100 | 150 |
| 6 | 6 | From tube 5 | 50 | 100 | 150 |
| 7 | 7 | From tube 6 | 50 | 100 | 150 |
| 8 (zero calibrator) | 8 | – | 0 | 150 | 150 |

Dash (—) = not applicable

**i** If you are preparing for more than one plate, adjust the volumes accordingly.

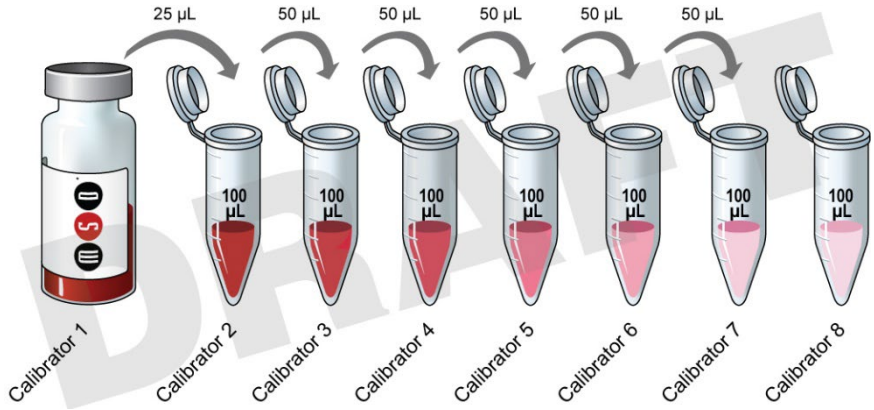


*Figure 2. Dilution schema for preparation of Calibrator Standards.*

**i** Thawed calibrator is stable for one day at 2–8 °C. It may also be stored frozen at ≤-70 °C and is stable through three freeze-thaw cycles. For the lot-specific concentration of each calibrator in the blend, refer to the COA supplied with the kit.

## Prepare Samples

To maximize *detection*, run samples undiluted (neat). If precise *quantitation* is desired, samples should be diluted in Diluent 6. An initial 4-fold dilution is recommended. Multiple dilutions may be required to determine the dilution at which concentration measurements fall within the linear (quantitative) range for each analyte. Samples should be heated for 1 hour at 60 °C to degrade endogenous antigens. Samples can be heat-treated in advance and stored frozen prior to testing. Controls and calibrators should not be heat treated.

## Prepare Controls

Two multi-analyte controls are provided at 1X. Add 30 µL per well of each control to the polypropylene plate.

## Prepare Antigen Mixtures

Important: Antigen vials should be thawed on wet ice. The diluted antigen mixtures should be prepared on ice and used immediately.

### Prepare Capture Antigen Mixture

- ❑ Add 80 µL of each capture antigen into a tube containing 3,680 µL of Diluent 100 on wet ice. Do not combine antigen stocks prior to addition to Diluent 100. Individual antigens are stable to at least one freeze-thaw cycle. Avoid multiple freeze-thaws of antigens.

### Prepare Detection Antigen Mixture

- ❑ Add 80 µL of each detection antigen into a tube containing 3,680 µL of Diluent 100 on wet ice. Do not combine antigen stocks prior to addition to Diluent 100. Individual antigens are stable to at least one freeze-thaw cycle. Avoid multiple freeze-thaws of antigens.

## MSD GOLD Read Buffer B

MSD provides MSD GOLD Read Buffer B ready for use. Do not dilute.

# Summary Assay Protocol

**Note:** Follow **Reagent Preparation** before beginning this assay protocol.

Prepare the assay mixture (antigens combined with samples, calibrators, or controls) in a 96-well polypropylene round bottom plate as a single mix per sample, calibrators, or control. Use the appropriate volumes for the number of replicates as described in Table 6.

- After the assay mixture has been prepared, transfer 50 µL of assay mixture per sample, calibrators, or control from the propylene plate to the appropriate number of replicate wells in the MSD assay plate.
- Add reagents in the sequence listed. Calibrator, controls, and samples should be added immediately to the antigen mixture to minimize assay background and non-specific signals.
- If multiple polypropylene plates are required, work with only one polypropylene plate at a time. Do not add detection antigen to the second plate until calibrator, controls, and samples are added to the first plate.
- If blank wells are run, add 30 µL of Diluent 6.

*Table 6. Example for preparation of singlicate wells in the polypopylene plate transferred to singlicate, duplicate, triplicate, or quadruplicate wells into the assay plate*

| Component | Dilution Factor | Singlicate (µL) | Duplicate (µL) | Triplicate (µL) | Quadruplicate (µL) |
|---|---|---|---|---|---|
| Capture Antigen Mix | 2.5 | 40 | 60 | 80 | 100 |
| Detection Antigen Mix | 2.5 | 40 | 60 | 80 | 100 |
| Calibrator, Controls and Samples | 5 | 20 | 30 | 40 | 50 |
| | **Total** | 100 | 150 | 200 | 250 |

Typical protocol for singlicate polyproplyene plate wells transferred to duplicate assay plate wells:

STEP 1: Assemble the Assay in a 96-well polypropylene U-bottom plate

- ❑ Add 60 µL of the capture antigen mix to each well of the plate.
- ❑ Add 60 µL of the detection antigen mix to each well of the plate.
- ❑ Add 30 µL of samples, calibrator, or controls.
- ❑ Seal the plate with an adhesive plate seal and incubate at room temperature with shaking (~700 rpm) for 60 minutes.
- ❑ Each well of the polypropylene plate contains 150 µL of assay mixture. This is sufficient volume to prepare duplicate replicates of 50 µL in the assay plate. The assay mixture can be scaled to prepare more or fewer replicates (Table 6).

STEP 2: Prepare the assay plate

- ❑ Add 50 µL of the reaction mixture from each well of the polypropylene plate into two wells (duplicates) of the assay plate.
- ❑ Seal the plate with an adhesive plate seal and incubate at room temperature with shaking (~700 rpm) for 90 minutes.

STEP 3: Wash and Read

- ❑ Wash the assay plate 3 times with at least 150 µL/well of Wash Buffer.
- ❑ Add 150 µL of MSD GOLD Read Buffer B to each well. Directly analyze the plate on an MSD instrument.

# Assay Characteristics

## Calibration Curves

For each plate, a calibration curve was generated by fitting signals from 7 calibrators and a blank to a 4-parameter logistic (4PL) model with 1/Y² weighting. Calibrators were prepared by serial dilution of the recommended top calibrator concentration, with the range designed to ensure the lowest calibrator fell below the expected LLOQ to improve accuracy at low analyte concentrations. Representative calibration curves from one SECTOR kit lot are presented in Figure 3.

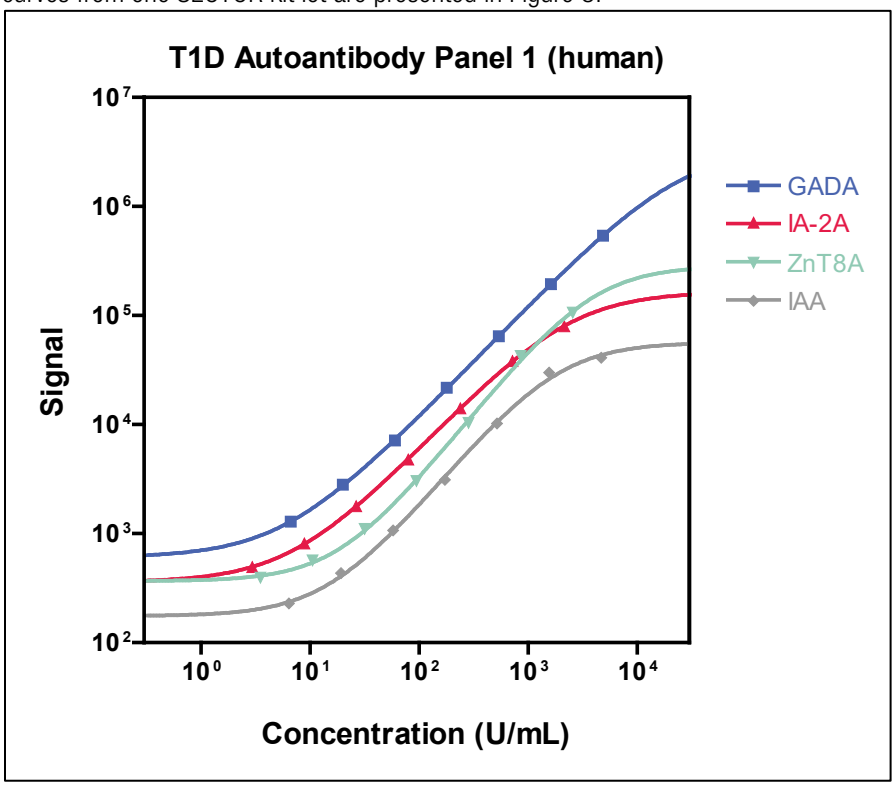


*Figure 3. Typical calibration curves for assays in the V-PLEX T1D Autoantibody Panel 1 Kit.*

## Sensitivity

The lower limit of detection (LLOD) is a calculated concentration corresponding to the signal 2.5 standard deviations above the background (zero calibrator). The LLOD shown below was calculated based on 36 runs.

The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) are established by measuring independent samples near the bottom and top of the calibration range, respectively. Each limit is set at the concentration where the %CV of back-calculated concentration value is less than 25% and concentration recovery is within 75–125% of the nominal value. Both limits were established across multiple kit lots, are verified for each kit lot, and are reported on the lot-specific Certificate of Analysis available at www.mesoscale.com.

The dynamic range of each assay spans from the LLOD to the ULOQ, typically covering 2–4 log units. The quantitative range, defined as the interval between the LLOQ and ULOQ, typically spans 2–3 log units.

*Table 7. LLOD, LLOQ, and ULOQ for each analyte in the T1D Autoantibody Panel 1 Kits*

| | Median LLOD (U/mL) | LLOQ (U/mL) | ULOQ (U/mL) | Range (U/mL) |
|---|---|---|---|---|
| GADA | 0.40 | 9.0 | 2,000 | 0.40 – 2,000 |
| IA-2A | 0.55 | 7.0 | 900 | 0.55 – 900 |
| IAA | 2.8 | 18 | 1,200 | 0.2.8 – 1,200 |
| ZnT8A | 1.6 | 16 | 800 | 1.6 – 800 |

## Precision

Precision was evaluated using the T1D Autoantibody Panel 1 (human) Controls 1 and 2. Analyte levels were measured from quadruplicate replicates from 36 runs performed by 3 operators over two days. The results are shown below. While a typical specification for precision is a concentration CV of less than 20% for controls on intra-run, inter-run and inter-lot CVs, for this panel, however, the data shows that all CVs for all assays are below 10%.

Average intra-run %CV is the average %CV of the control replicates within individual runs. Inter-run %CV is the variability of controls across 36 runs, averaged across three kit lots. Inter-lot %CV is the variability of controls across three kit lots (36 runs).

*Table 8. Intra-run and inter-run %CVs for each analyte in the T1D Autoantibody Panel 1 Kits*

| | Control | Average Conc. (U/mL) | Average Intra-run %CV | Inter-run %CV | Inter-lot %CV |
|---|---|---|---|---|---|
| GADA | Control 1 | 405 | 4.2% | 6.4% | 7.3% |
| | Control 2 | 60 | 4.5% | 7.6% | 8.0% |
| IA-2A | Control 1 | 305 | 4.2% | 5.6% | 8.4% |
| | Control 2 | 33 | 3.9% | 6.3% | 8.1% |
| IAA | Control 1 | 510 | 3.3% | 4.5% | 5.7% |
| | Control 2 | 118 | 4.2% | 5.1% | 6.7% |
| ZnT8A | Control 1 | 421 | 3.4% | 4.5% | 5.6% |
| | Control 2 | 127 | 3.7% | 5.2% | 6.2% |

## Stability

The reconstituted calibrator, controls, and diluents were tested for freeze-thaw stability. Results (not shown) demonstrated that the calibrator, controls, and Diluent 6 can go through three freeze-thaw cycles without significantly affecting the assay's performance. Once thawed, the multi-analyte calibrator is stable for one day at 2–8 °C.
Kit validation study includes a real-time stability study with scheduled performance evaluations of complete kits for up to 60 months from manufacture.

## Calibration

GADA and IA-2A were calibrated against the WHO Reference Reagent, Islet Cell Antibodies, NIBSC code: 97/550 in Units of biological activity per mL (U/mL). ZnT8A and IAA are assigned units per mL.

## Assay Components

### Calibrators

Calibrators are recombinant monoclonal antibodies that are detected by the human antigens. The T1D Autoantibody Panel 1 (human) calibrator blend consists of the following antibodies:

*Table 9. Antibodies used in the calibrators*

| Calibrator | Source |
|---|---|
| GADA | Recombinant Monoclonal Anti-GAD2/GAD-65 Antibody |
| IA-2A | Recombinant Monoclonal Anti-IA-2/R-PTP-N Antibody |
| ZnT8A | Recombinant Monoclonal Anti-ZnT8 Antibody |
| IAA | Recombinant Monoclonal Insulin Antibody |

### Antigens

*Table 10. Antigen expression systems*

| | Expression System | | |
|---|---|---|---|
| Antigen | Capture | Detection | Assay Generation |
| GAD2/GAD-65 | HEK-293 | HEK-293 | A |
| IA-2/P | Insect | Insect | A |
| ZnT8 | E. coli | E. coli | A |
| Insulin | Yeast | Yeast | A |

# References


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