Simple Plex™ Assay Validation Summary

Calibration Curve

- **Description:** A minimum of 4 independent standard curves must be run by each of four users using two different lots of reagents (i.e. diluent, GNR capture, and biotinylated detect lots).
- **Dynamic range:**
  - LOD (limit of detection) using a minimum of 15 zero replicates = 3 stdev of the zero RFU added to the average RFU value and back fit to the curve.
  - A common method of calculating the limits of quantitation (LLOQ and ULOQ) starts by measuring the signal (RFU) for a series of "known concentrations" over the expected dynamic range of the assay, then finding the best fit curve which represents the entire data set. To identify the LLOQ and ULOQ, a CV of the replicate calculated concentration values at each "known concentration" is determined. Thus a table containing CVs for each "known concentration" is formed and the limits are found by simply identifying, in the case of the LLOQ, the lowest concentration with a CV below 20%, and for the ULOQ, the highest concentration with a %CV less than 20%.
- **Acceptance criteria:**
  - Sensitivity (defined as the concentration at which CVs are <20%) must be within an appropriate range of expected sample values.
  - Percent recovery = calculated concentration / expected concentration * 100.
  - 80–120% recovery of curve points between LLOQ and ULOQ.

Inter/Intra Assay Precision

- **Description:** Controls concentrations should fall within the limits of quantitation of the standard curve. A minimum of one control cartridge is run for each control level to assess intra-assay CV (n = 16). The CV between cartridges can be used to assess the inter-assay variability.
- **Acceptance criteria:** Intra-assay CVs at each control level should be ≤10%. Inter-assay CVs at each control level should be ≤15%.

Correlation with Quantikine® ELISA Kits from R&D Systems

- **Description:** Multiple samples will be analyzed to compare both platforms overlapping the dynamic range.
- **Acceptance criteria:** The slope should be between 0.9–1.1 and an R^2 value greater than 0.9.

Simple Plex Assay Validation Summary

Accuracy (Spike and Recovery)

- **Description:** Unspiked + 3 spiked concentrations for 4 individual serum, 8 individual plasma (4 EDTA, 4 Heparin), 8 individual platelet poor plasma (where appropriate).
- **Percent recovery:** (Observed Concentration - Endogenous) / Measured Control Spike Concentration * 100.
- **Acceptance criteria:**
  - Mean recovery of all samples at all concentrations between 75–125%.
  - Individual sample mean recovery at all concentrations between 70–130% for all samples.

Parallelism and/or Linearity

- **Description:** Test a minimum of 4 serum, 4 plasma EDTA, and 4 plasma Heparin serially diluted 1:2, 1:4, 1:8 and 1:16.
- **Acceptance criteria:** Percent recovery for all dilutions above minimum required dilution (MRD) and between LLOQ and ULOQ should have 75–125% recovery.

Cross-reactivity, Specificity, and Interference

- **Description:** Assess cross-reactivity or interference in similar analytes, soluble receptors, and other factors such as soluble binding partners within a species. Samples are tested for cross-reactivity and interference at levels 10X the ULOQ in the Sample Diluent or a mid-level Control respectively. Analyze the potential for interference of substances such as hemoglobin, bilirubin, and triolein.
- **Acceptance criteria:** Samples that cross-react or interfere at 10X the ULOQ of the assay are repeated at lower dilutions to more accurately determine the cross-reactivity or interference. Samples generating values of ≥1% cross-reactivity are reported in the specification sheet. Factors that cause the control to fall outside a 3 SD range of the accepted value are considered to interfere in the assay and are reported in the specification sheet.

R&D SYSTEMS™
a bio-techne brand

proteinsimple

Toll-free: (888) 607-9692
Tel: (408) 510-5500
Fax: (408) 510-5599
info@proteinsimple.com
proteinsimple.com

D41-0044-001 Rev A