The standard curve has been successfully modeled using a **4-parameter logistic (4PL)** regression. The fit looks good, with the binding percentage decreasing in a sigmoidal shape as the cortisol concentration increases — exactly what we expect in a competitive ELISA.

Next:

1. **Precision** – by examining %CV of replicate wells.
2. **Accuracy** – using backfitted values of known standards.
3. **Recovery** – using the spiked vs. non-spiked sample data from sample\_info\_test3\_091524.csv.

Let’s proceed by calculating **intra-assay precision** and examining **accuracy metrics** based on the standard values.

**✅ ELISA Performance Summary – Test 3 (091524)**

Here’s a breakdown of the results from your ELISA run, focusing on **precision**, **accuracy**, and soon, **recovery**.

**📌 1. Precision (Intra-assay)**

Precision is evaluated by the **%CV (coefficient of variation)** between replicates of the same sample:

* **Mean %CV:** **11.36%**
* **Best %CV (lowest):** **0.57%**
* **Worst %CV (highest):** **98.00%**

💡 A %CV under **15%** is generally acceptable for most ELISA assays. Values over 20% should be flagged for review (e.g., potential pipetting error, evaporation, or static issues).

**📌 2. Accuracy (Standard Curve Backfit)**

Accuracy reflects how close your measured standard concentrations (via backfit) were to their expected nominal values:

* **Mean Accuracy:** **99.74%**
* **Minimum Accuracy:** **92.4%**
* **Maximum Accuracy:** **107.15%**

✅ These values show excellent accuracy, with backfitted values deviating by less than ±10% from the true concentration across the curve.

**🔬 Recovery Assessment – Test 3 (091524)**

The recovery values from your spiked samples were calculated based on a presumed spike-in of **800 pg/mL** cortisol into each test sample. Here’s the summary:

**📌 3. Recovery (Spiked vs. Non-Spiked Samples)**

* **Mean Recovery:** **238.53%**
* **Minimum Recovery:** **73.36%**
* **Maximum Recovery:** **377.38%**
* **Number of Recovery Pairs:** **9**

**🧪 Interpretation**

* The **very high average recovery (238%)** suggests either:
  + **Overestimation of cortisol** in spiked samples, or
  + **Underestimation in baseline (non-spiked)** samples, or
  + **Incorrect spike-in assumptions**, such as:
    - Miscalculated actual volume/concentration added
    - Loss or concentration during processing
* One recovery as low as ~73% is borderline acceptable.
* **Ideal range:** Recovery between **80–120%** is typically acceptable in bioanalytical assays.

**✅ Summary of ELISA Test 3 Performance**

| **Metric** | **Result** | **Interpretation** |
| --- | --- | --- |
| **Precision** | Mean %CV = **11.36%** | Acceptable (<15% for most wells) |
| **Accuracy** | Mean = **99.74%** | Excellent (close to expected values) |
| **Recovery** | Mean = **238.53%** | High – indicates spike recovery inconsistency |