AAV08-EN.01

# AAV8 Titration ELISA Kit

# (Enzyme-Linked Immunosorbent Assay)

**Catalog Number：AAV-A008**

**Pack Size: 96 tests**

**IMPORTANT: Please carefully read this manual before performing your experiment.**

***For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedure***

## INTENDED USE

AAV8 Titration ELISA Kit was developed for the detection and quantitative determination of AAV8 capsid titration in AAV gene therapy product preparation processing. It is intended for research use only (RUO).

## BACKGROUND

The adeno-associated virus (AAV) is a small (25 nm), non-enveloped virus of the parvorder family, including 12 different AAV serotypes. In the parvorder family it belongs to the genus depend parvovirus, because it needs the presence of a helper virus for replication and assembly. The icosahedral AAV capsid composed of the capsid proteins VP1, VP2 and VP3 contains a linear, single-stranded DNA genome of 4.7 kb.

To support the development of gene therapy, ACROBiosystems independently developed AAV8 Titration ELISA Kit via rigorous methodological validation, which is used for detection of AAV8 capsid titration in samples.

## PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of AAV8 capsid by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-AAV8 Antibody. Firstly, add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Anti-AAV8 Antibody to the plate and form Antibody-antigen-biotinylated antibody complex, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. At last, load the substrate into the wells and monitor solution color from blue to yellow. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm and 630 nm. The OD Value reflects the amount of AAV8 capsid bound.

## PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Do not use reagents past their expiration date.

3. Do not mix or substitute reagents with those from other kits or other lot number kits.
4. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay. If cell supernatant samples need step dilution, except for the final dilution with diluent, other intermediate dilutions can be in cell culture medium.
5. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage.
6. This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

## MATERIALS PROVIDED

**Table1. Materials provided**

| Catalog | Components | Size (96 tests) | Format | Storage | |
|---|---|---|---|---|---|
| | | | | Unopened | Opened |
| AAV08-C01 | Pre-coated Anti-AAV8 Antibody Microplate | 1 plate | Solid | 2-8°C | 2-8°C |
| AAV08-C02 | AAV8 Standard | 1.4E+10 capsids | Powder | 2-8°C | -70°C |
| AAV08-C03 | Biotin-Anti-AAV8 Antibody | 15 μg | Powder | 2-8°C | -70°C |
| AAV08-C04 | Streptavidin-HRP | 50 μL | Liquid | 2-8°C, avoid light | 2-8°C, avoid light |
| AAV08-C05 | 10×Washing Buffer | 50 mL | Liquid | 2-8°C | 2-8°C |
| AAV08-C06 | 2×Dilution Buffer | 50 mL | Liquid | 2-8°C | 2-8°C |
| AAV08-C07 | Substrate Solution | 12 mL | Liquid | 2-8°C, avoid light | 2-8°C, avoid light |
| AAV08-C08 | Stop Solution | 7 mL | Liquid | 2-8°C | 2-8°C |

***Note:*** *It is recommended that Streptavidin-HRP be centrifuged briefly before use to deposit liquid from the tube wall or cap to the bottom of the tube.*

## SRORAGE

1. Unopened kit should be stored at 2°C -8°C upon receiving.

2. The opened kit should be stored per Table 1. The shelf life is 30 days from the date of opening.

***Note:*** *a. Do not use reagents past their expiration date.*

*b. Find the expiration date on the outside packaging.*

## REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or multi-channel micropipettes and pipette tips: need to meet 10 μL, 300 μL, 1000 μL injection requirements;

37ºC Incubator;

Single or dual wavelength microplate reader with 450 nm and 630 nm filter;

Tubes: 1.5mL, 10mL;

Timer;

Reagent bottle;

Deionized or distilled water.

## REAGENT PREPARATION

Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in an 37℃ incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

According to Table 2, prepare the provided lyophilized product into a storage solution, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoiding vigorous shaking or vertexing. The reconstituted storage solution should be stored at -70°C. AAV08-C02 is recommended not to freeze-thaw more than 1 times, the packing specification shall not be less than 12 μL. AAV08-C03 is recommended not to freeze-thaw more than 1 times, the packing specification shall not be less than 4μg.

***Note:****Considering inevitable minor quantitation variations between protein batches, it is also reasonable to generate the standard curve with specific lot of proteins used for current production for even better accuracy.*

**Table 2. Preparation method**

| ID | Components | Size (96 T) | Storage solution concentration. | Reconstituted Buffer Vol. |
|---|---|---|---|---|
| AAV08-C02 | AAV8 Standard | 1.4E+10 capsids | 2.8E+11 capsids/mL | 50 µL 1×PBS |
| AAV08-C03 | Biotin-Anti-AAV8 Antibody | 15 µg | 150 µg/mL | 100 µL Water |

## RECOMMENDED SAMPLE PREPARATION

### 1. Working Solution Preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

1.3 Preparation of Biotin-Anti-AAV8 Antibody working fluid:

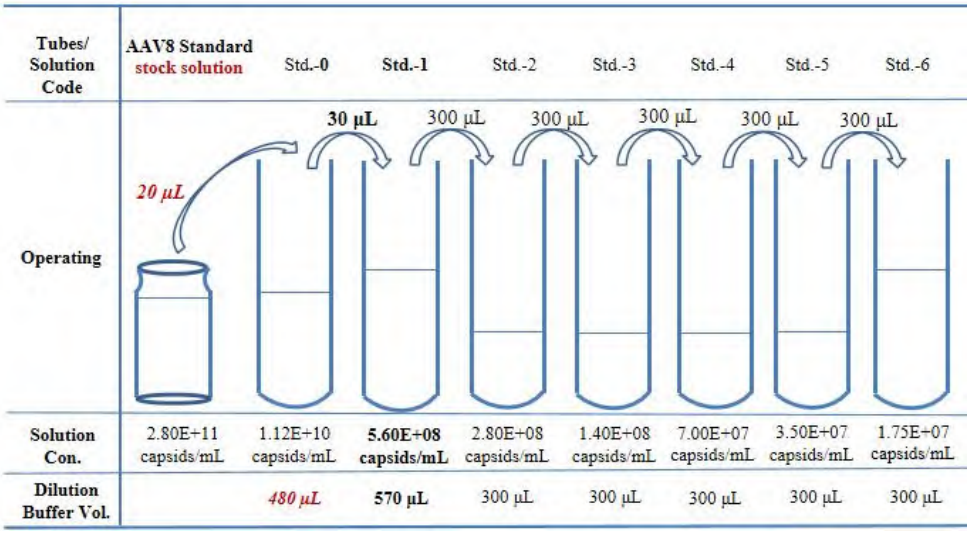
Dilute Biotin-Anti-AAV8 Antibody to 0.2 µg/mL with 1×Dilution Buffer. Please prepare it for one-time use only.

1.4 Preparation of Streptavidin-HRP working fluid:

Dilute Streptavidin-HRP at 1:2000 with 1×Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

### 2. Preparation of Standard curve

The concentration of the reconstituted AAV8 Calibrator (AAV08-C02) is 2.8E+11 capsids/mL， prepare (Std.-0) by diluting 20 µL the reconstituted AAV8 Calibrator into 480 µL Sample Dilution Buffer, mix gently well. Then prepare the highest concentration of standard curve, Std.-1 (5.60E+08 capsids/mL), by diluting 30 µL Std.- 0 into 570 µL Sample Dilution Buffer. Prepare 1:1 serial dilution for the standard curve as follows: Pipette 300 µL of Sample Dilution Buffer into each tube. Make sure to mix well every time. Sample Dilution Buffer serves as blank.




### 3. Add Samples

Add 100 µL Calibrator and samples to each well. For blank Control wells, please add 100 µL Dilution Buffer.

***Note**: It is recommended to set double holes for samples and standard curves to be tested.*

### 4. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

### 5. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 30 s, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

### 6. Add Biotin-Anti-AAV8 Antibody

For all wells, add 100 µL Biotin-Anti-AAV8 Antibody (dilute to 0.2 µg/mL) working solution. Please prepare it for one-time use only.

### 7. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

### 8. Washing

Repeat step 5.

### 9. Add Streptavidin-HRP

For all wells, add 100 µL Streptavidin-HRP (dilute at 1:2000) working solution. Please prepare it for one-time use only, avoid light.

**10. Incubation**

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

**11. Washing**

Repeat step 5.

**12. Substrate Reaction**

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

**13. Termination**

Add 50 µL Stop Solution to each well and tap the plate gently to allow thorough mixing.

***Note:*** *The color in the wells should change from blue to yellow.*

**14. Data Recording**

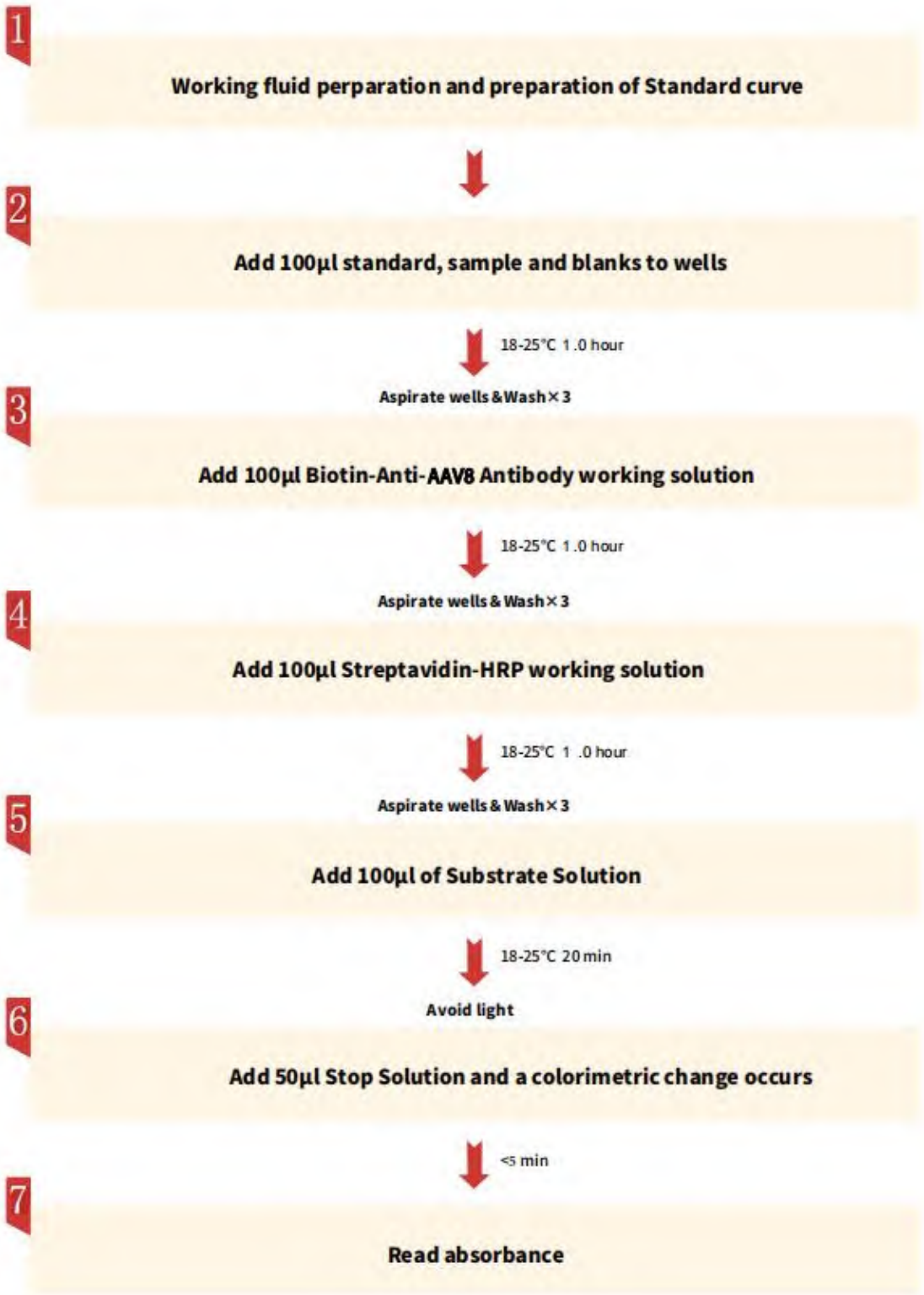
Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 5 minutes.

***Note****: To reduce the background noise, subtract the value read at* $OD_{450nm}$ *with the value read at* $OD_{630\ nm}$.

## CALCULATION OF RESULTS

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (OD).
2. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.
3. Normal range of Standard curve: $R^2 \geq 0.9900$.
4. Detection range: 1.75E+07 capsids/mL-5.60E+08 capsids/mL. If the OD value of the sample to be tested is higher than 5.60E+08 capsids/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 1.75E+07 capsids/mL, the sample should be reported.

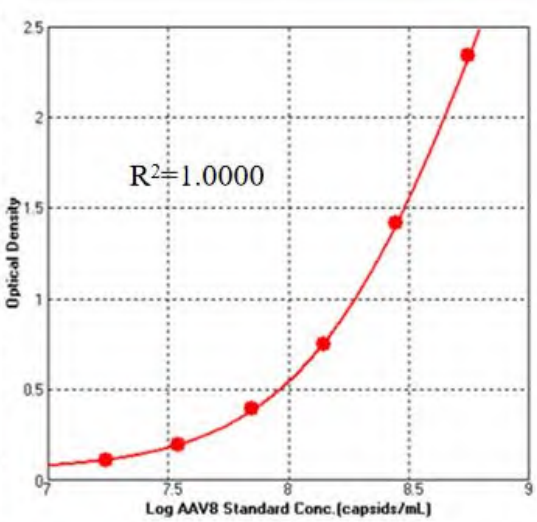
# QUICK GUILD


1
Working fluid perparation and preparation of Standard curve
2
Add 100µl standard, sample and blanks to wells
18-25°C 1.0 hour
Aspirate wells & Wash × 3
3
Add 100µl Biotin-Anti-AAV8 Antibody working solution
18-25°C 1.0 hour
Aspirate wells & Wash × 3
4
Add 100µl Streptavidin-HRP working solution
18-25°C 1 .0 hour
Aspirate wells & Wash × 3
5
Add 100µl of Substrate Solution
18-25°C 20 min
Avoid light
6
Add 50µl Stop Solution and a colorimetric change occurs
<5 min
7
Read absorbance

## TYPICAL DATA

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.

| Standard (capsids/mL) | O.D.-1 | O.D.-2 | Average | Corrected |
|---|---|---|---|---|
| 5.60E+08 | 2.384 | 2.419 | 2.402 | 2.337 |
| 2.80E+08 | 1.595 | 1.365 | 1.480 | 1.416 |
| 1.40E+08 | 0.839 | 0.793 | 0.816 | 0.752 |
| 7.00E+07 | 0.452 | 0.459 | 0.456 | 0.391 |
| 3.50E+07 | 0.265 | 0.257 | 0.261 | 0.197 |
| 1.75E+07 | 0.192 | 0.158 | 0.175 | 0.111 |
| 0 | 0.062 | 0.067 | 0.064 | / |




## SENSITIVITY

The minimum detectable concentration of AAV8 is 3.27E+06 capsids/mL. The minimum detectable concentration was determined by adding twice standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

## PRECISION

1. Intra-assay Precision

Three samples of known concentration were tested ten times on one plate to assess intra-assay precision.

2. Inter-assay Precision

Three samples of known concentration were tested in three separate assays to assess inter-assay precision.

| | Intra-assay Precision | | | Inter-assay Precision | | |
|---|---|---|---|---|---|---|
| Sample | 1 | 2 | 3 | 1 | 2 | 3 |
| n | 10 | 10 | 10 | 3 | 3 | 3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Mean (capsids/mL) | 358.765 | 93.877 | 37.003 | 360.948 | 92.080 | 36.437 |
| SD | 15.908 | 2.463 | 2.146 | 3.783 | 1.883 | 0.491 |
| CV (%) | 4.4 | 2.6 | 5.8 | 1.0 | 2.0 | 1.3 |

***Note**: The example data is for reference only.*

## RECOVERY

Three AAV8 with different concentrations were tested to calculate the recovery rate.

| Sample(n=5) | Average% Recovery | Range % |
|---|---|---|
| High | 91.7 | 88.1-97.0 |
| Middle | 88.9 | 85.3-93.0 |
| Low | 89.4 | 81.8-96.8 |

## LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of AAV8 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

| | | Cell culture medium (DMEM) | Cell culture medium (1640) |
|---|---|---|---|
| 1:2 | Average Recovery (%) | 102.4 | 88.8 |
| | Range (%) | 97.3-105.9 | 82.8-92.1 |
| 1:4 | Average Recovery (%) | 100.0 | 93.8 |
| | Range (%) | 95.9-104.8 | 92.8-95.2 |
| 1:8 | Average Recovery (%) | 101.1 | 95.0 |
| | Range (%) | 93.8-106.4 | 92.0-97.7 |
| 1:16 | Average Recovery (%) | 101.2 | 99.6 |
| | Range (%) | 94.6-105.0 | 97.1-102.6 |

***Note**: The example data is for reference only.*

## SPECIFICITY

This assay recognizes AAV8 specifically. No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines.

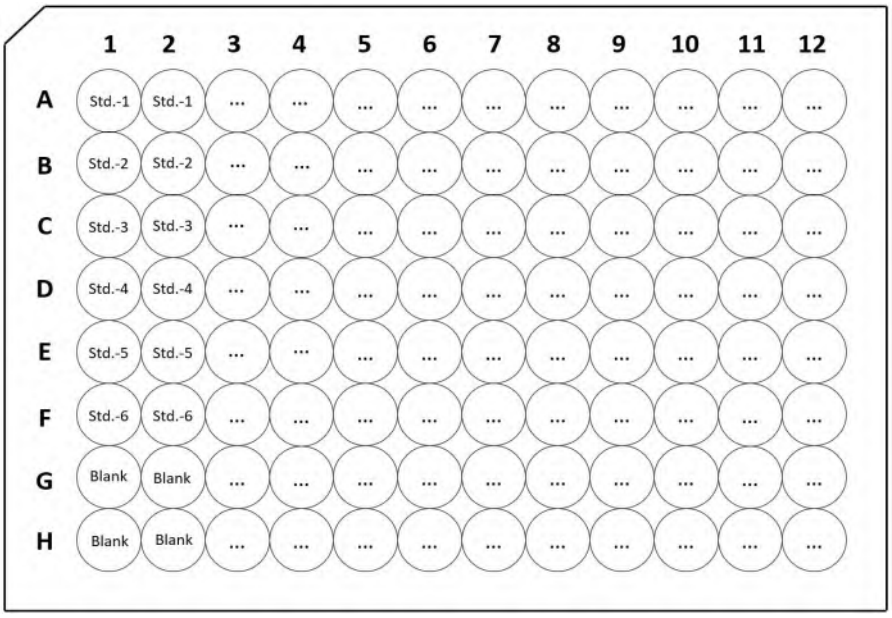
| Specificity | | |
|---|---|---|
| AAV1 | AAV2 | AAV3 |
| AAV5 | AAV6 | AAV9 |
| AAV dj | | |

## INTERFERING SUBSTANCES

Analysis of matrix effects of different additives on the AAV8 Titration ELISA.

| Additive/Matrix | Tolerated concentration |
|---|---|
| Pluronic F-68 | 0.05% |
| MgCl2 | 50 mM |
| Triton X-100 | 0.5% |
| Tween20 | 0.5% |
| EDTA | 10 mM |
| NaCl | 1 M |

## PLATE LAYOUT



***Note:*** *Blank is a Blank Dilution Buffer hole.*

## TROUBLESHOOTING GUIDE

| Problem | Cause | Solution |
|---|---|---|
| **Poor standard curve** | * Inaccurate pipetting | * Check pipettes |
| **Large CV** | * Inaccurate pipetting<br>* Air bubbles in wells | * Check pipettes<br>* Remove bubbles in wells |
| **High background** | * Plate is insufficiently washed<br>* Contaminated wash buffer | * Review the manual for proper wash.<br>* Make fresh wash buffer |
| **Very low readings across the plate** | * Incorrect wavelengths<br>* Insufficient development time | * Check filters/reader<br>* Increase development time |
| **Samples are reading too high, but standard curve looks fine** | * Samples contain cytokine levels above assay range | * Dilute samples and run again |
| **Drift** | * Interrupted assay set-up<br>* Reagents not at room temperature | * Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of theassay<br>* Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts |