Luminex Xponent: Plate- level Quality Control

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Aim

This code aims to do a plate- level quality control analysis for Luminex studies through bead counts, Background MFI, and Standard curve analysis.

R Markdown

This is an R Markdown document, areas that between the `{r}` frames that are require manual entries as described in the **bolded text**. When entries are completed, press the *knit* button at the top of the page.

Dependencies

- R version 4.3.1 (2023-06-16)
  - `tidyverse` (v. 2.0.0)
  - `here` (v. 1.0.1)

Required Inputs

1. Confirm working directory:

This is the project folder where the project is found. All folders and files added to the code will be within this folder. If needed you can set the working directory using the `setwd()` function.

Working directory: /Users/sahal/Documents/R Projects/RTSS_Kisumu_Schisto

2. Choose file (input required):

Chose the raw plate file from working directory by adding what folder within (in the quotes). In the example below, the plate csv is in the `Project/data/raw/luminex/` folder.

3. Set minimum bead count:

Set the **minimum bead count** as `min_beadcount`. In this example, the standard we have set here is `50`. All bead counts <50 will be identified.

4 Define the `file_path`:

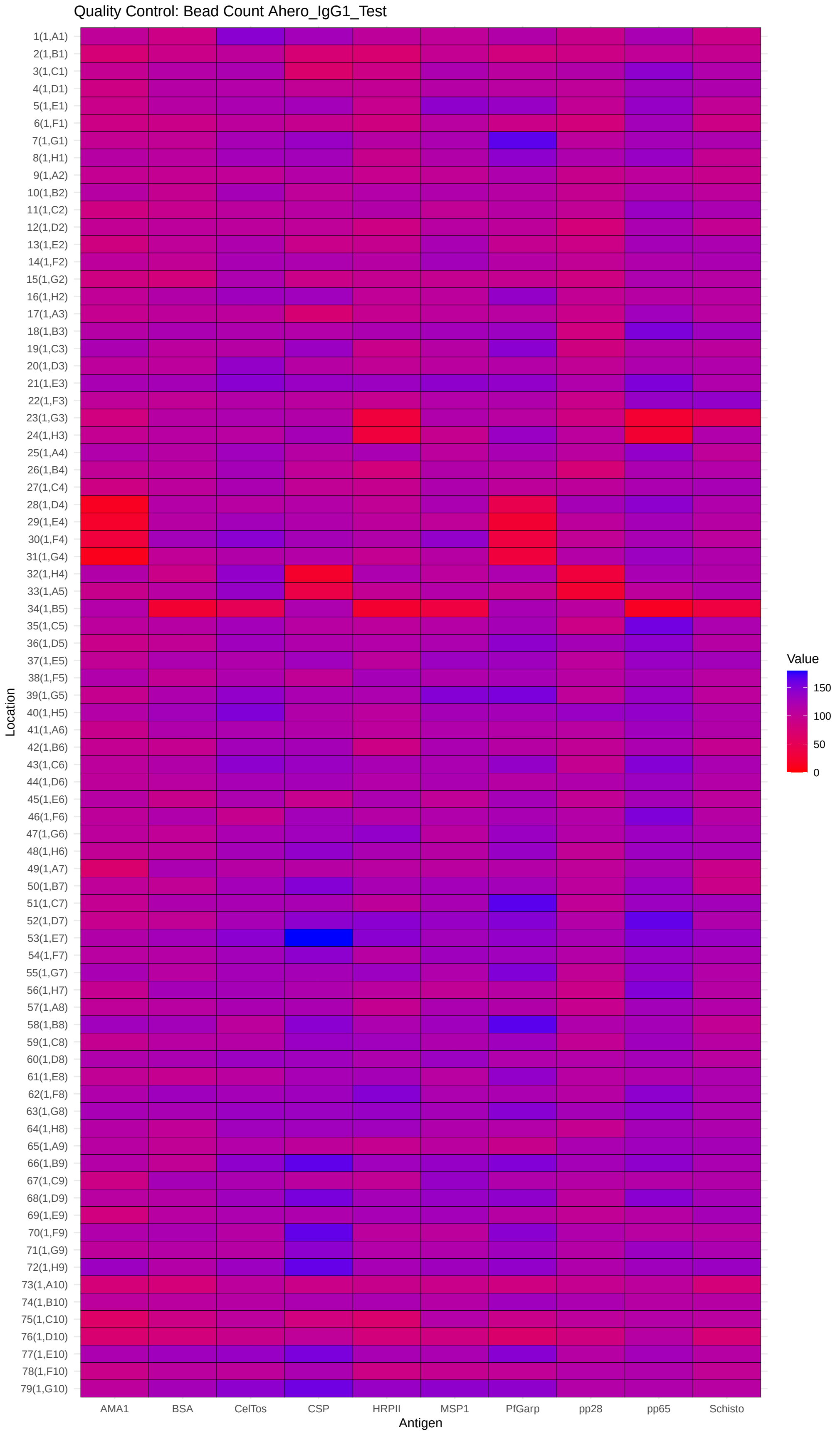
For example, here "Project/data/qc" folder is where the *PlateName_beadqc_df.csv will be saved*

```r
# Choose the input file
input_file <- "data/raw/luminex/Ahero_IgG1_Test.csv"
# Minimum bead count for analysis
min_beadcount <- 50

#Output file path
file_path <- "data/qc"
```

Quality Control

Bead count (Input required)

```
## The bead QC report has been exported to data/qc/Ahero_IgG1_Test_beadqc.png
```



Quality Control: Bead Count Ahero_IgG1_Test

List of wells/analytes <50 beads

This code results in a list of wells, sample IDs, and analytes that are less than the minimum bead count set. This file is exported to `file_path` (chosen above) as *PlateName_beadqc_list.csv*. Values in this list will be `Na` in the Median MFI dataframe. `file_path`: data/qc (this can be edited in the **Bead Count** section)

```
## The bead QC list has been exported to data/qc/Ahero_IgG1_Test_beadqc_list.csv
```

```
##    Location      Sample  Column
## 1  28(1,D4)     RAM-007    AMA1
## 2  29(1,E4) RAC-007-T01    AMA1
## 3  30(1,F4) RAC-007-T07    AMA1
## 4  31(1,G4) RAC-007-T08    AMA1
## 5  32(1,H4)     RAM-008    pp28
## 6  33(1,A5) RAC-008-T01    pp28
## 7  23(1,G3) RAC-002-T07 Schisto
## 8  34(1,B5) RAC-008-T07 Schisto
## 9  23(1,G3) RAC-002-T07    pp65
## 10 24(1,H3) RAC-002-T08    pp65
## 11 34(1,B5) RAC-008-T07    pp65
## 12 34(1,B5) RAC-008-T07     BSA
## 13 34(1,B5) RAC-008-T07    MSP1
## 14 23(1,G3) RAC-002-T07   HRPII
## 15 24(1,H3) RAC-002-T08   HRPII
## 16 34(1,B5) RAC-008-T07   HRPII
## 17 34(1,B5) RAC-008-T07  CelTos
## 18 32(1,H4)     RAM-008     CSP
## 19 33(1,A5) RAC-008-T01     CSP
## 20 28(1,D4)     RAM-007  PfGarp
## 21 29(1,E4) RAC-007-T01  PfGarp
## 22 30(1,F4) RAC-007-T07  PfGarp
## 23 31(1,G4) RAC-007-T08  PfGarp
```

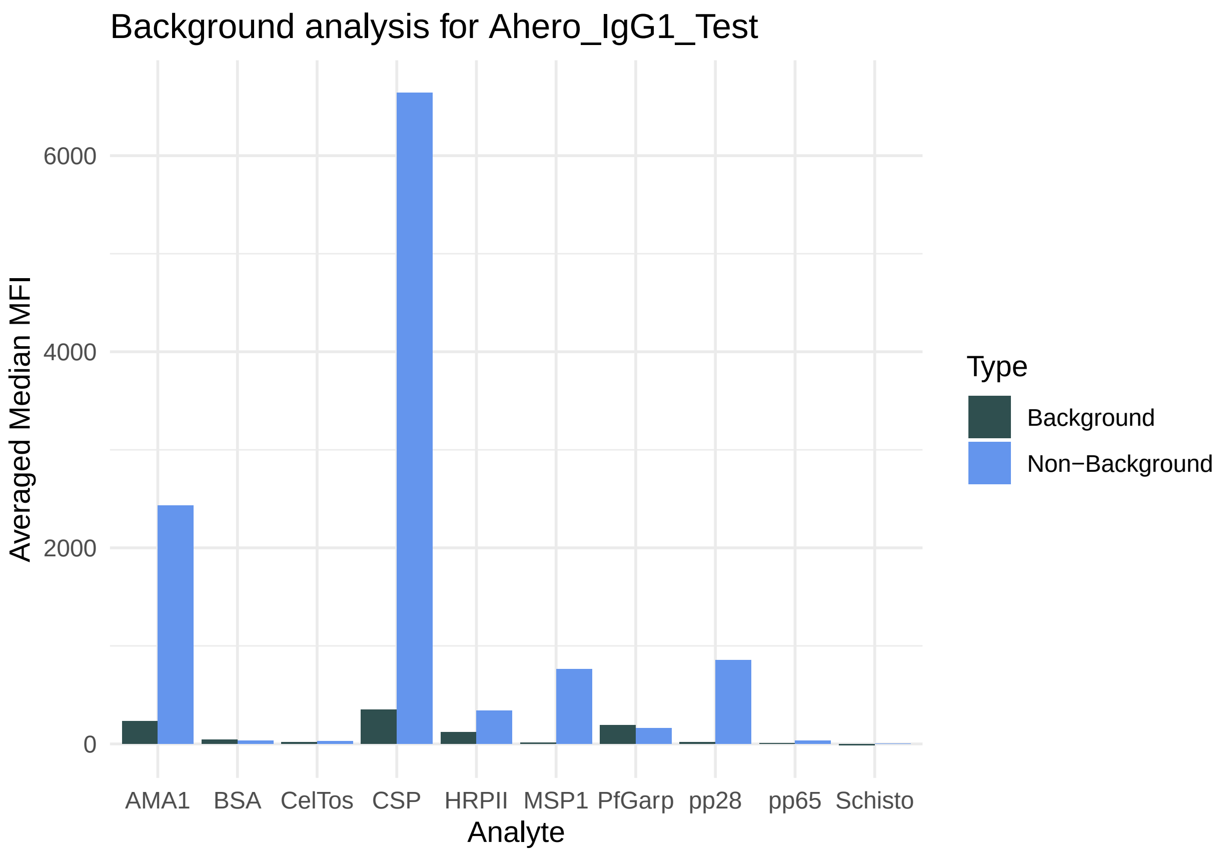
QC of median MFI data

This extracts the median MFI data, with all well-analyte combinations <50 beads/well replaced with *NA*. It completes the BSA MFI subtraction as well

note: negative values are kept

Background MFI

This code averages the median MFI values for all background and non-background samples. This requires all Background samples to have "Background" within the characters in the `Sample` column



Standard curve

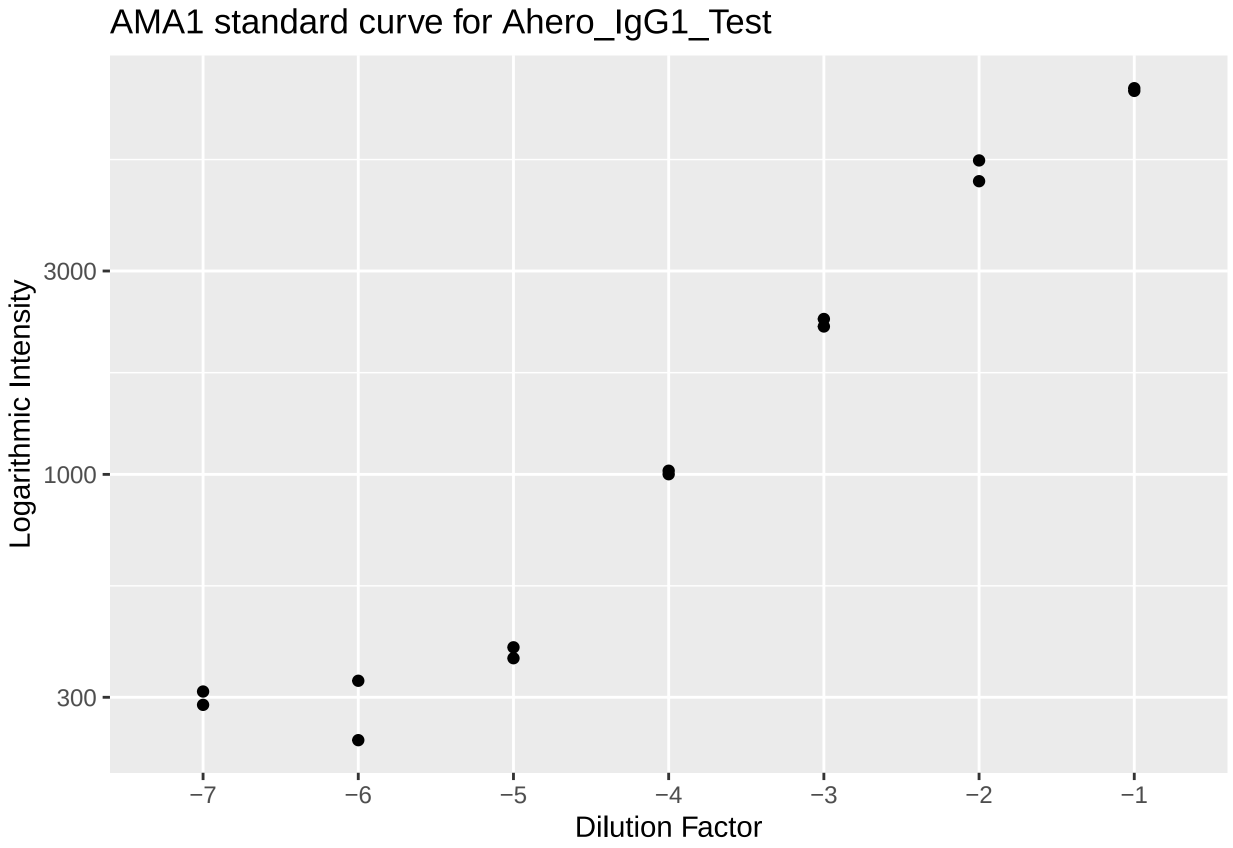
This code will attempt to create standard curves. This requires the samples have "Standard" within the characters in the `Sample` column. This will extraction `Dilution_Factor` as the number within the name (x -1).

For example: *Standard 2* will have a `Dilution_factor` of -2

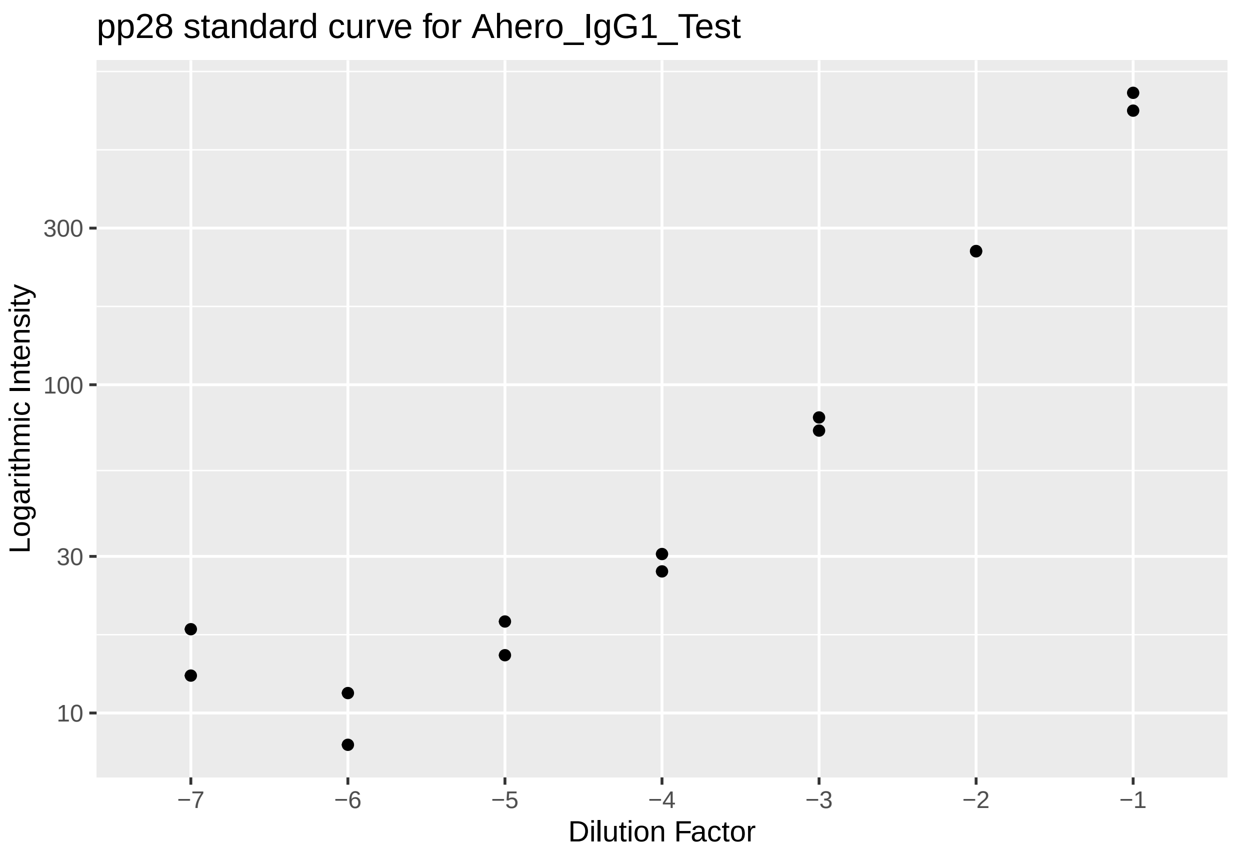
This code will run for all `analyte`

```
# No input required
    # Create a standards dataframe
    standards_df <- results_df %>%
      filter(str_detect(Sample, "Standard")) %>%
          # Extract dilution factor from the Sample column and make it negative
          mutate(Dilution_Factor = as.factor(-as.numeric(str_extract(Sample, "\\d+")))) %>%
          # Move Dilution_Factor column to the second position
          select(Sample, Dilution_Factor, everything())

    # Create Plot
        # plots_list
        plots_list <- lapply(analytes, function(analyte) {
          # Create Title
            title <- paste(analyte, "standard curve for", gsub(".csv", "", basename(input_file)))
          # Create plot for the current analyte
          plot <- ggplot(standards_df, aes(x = Dilution_Factor, y = !!sym(analyte))) +
            geom_point() +
            labs(title = title,
                 x = "Dilution Factor",
                 y = "Logarithmic Intensity") +
            scale_y_log10()  # Add log10 scale to y-axis

          return(plot)
        })


        # Display plots
        plots_list
```

```
## [[1]]
```



```
##
## [[2]]
```

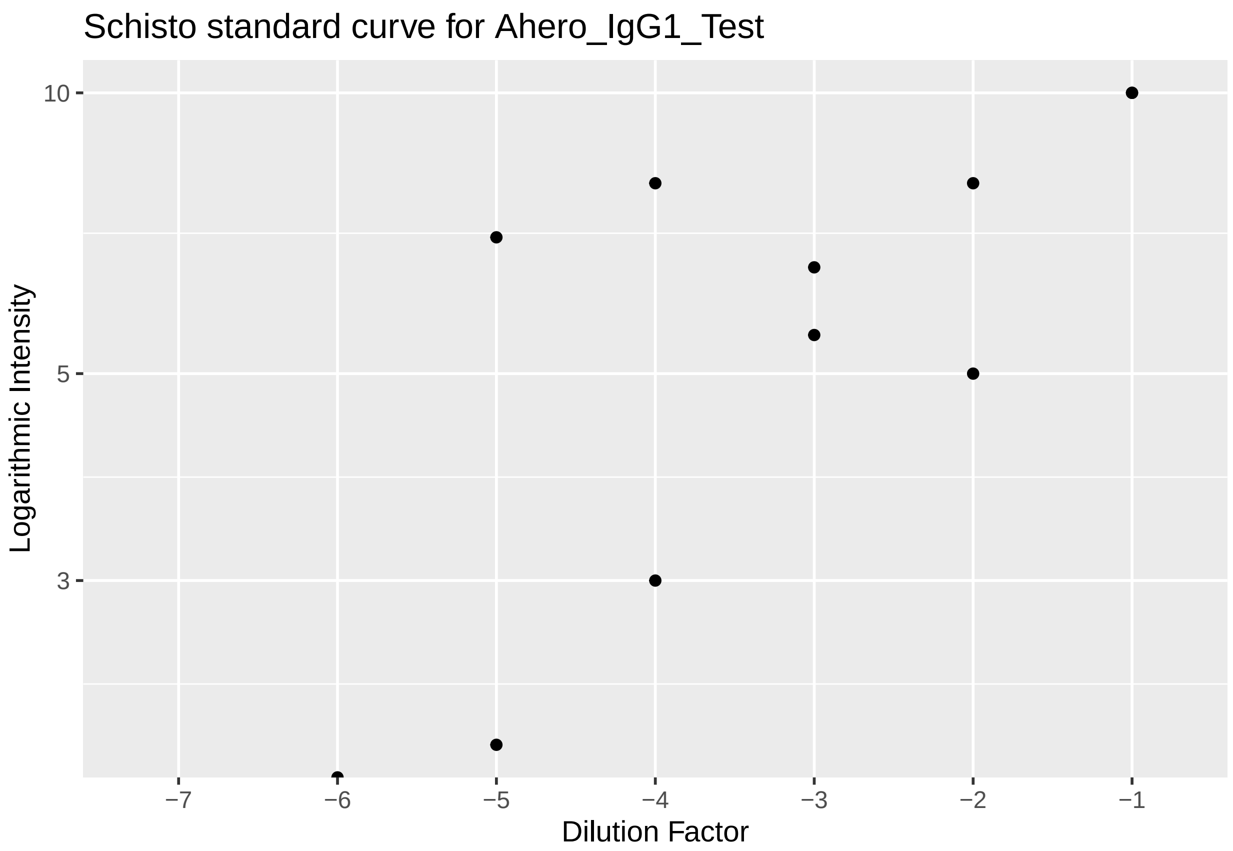


```
##
## [[3]]
```

```
## Warning in self$trans$transform(x): NaNs produced
```

```
## Warning: Transformation introduced infinite values in continuous y-axis
```

```
## Warning: Removed 3 rows containing missing values (‘geom_point()‘).
```

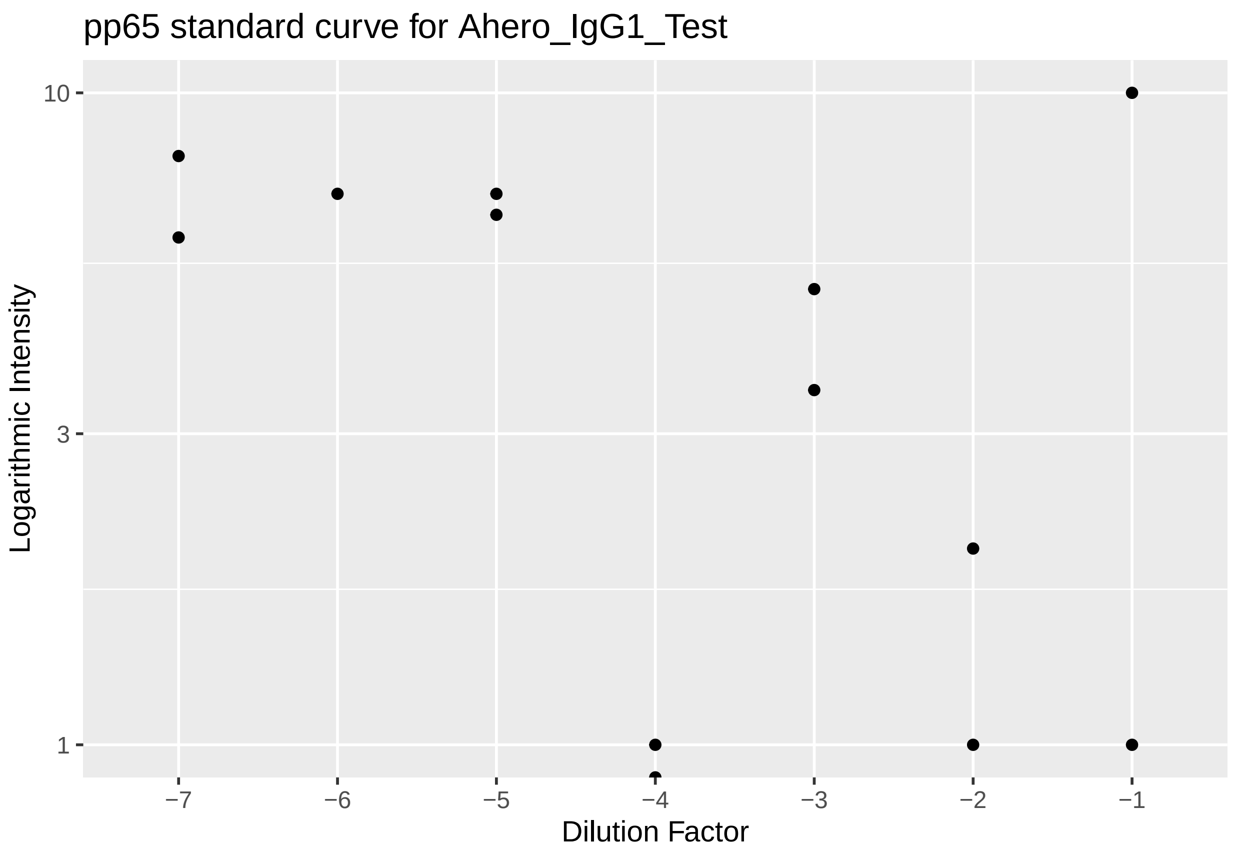


```
##
## [[4]]
```

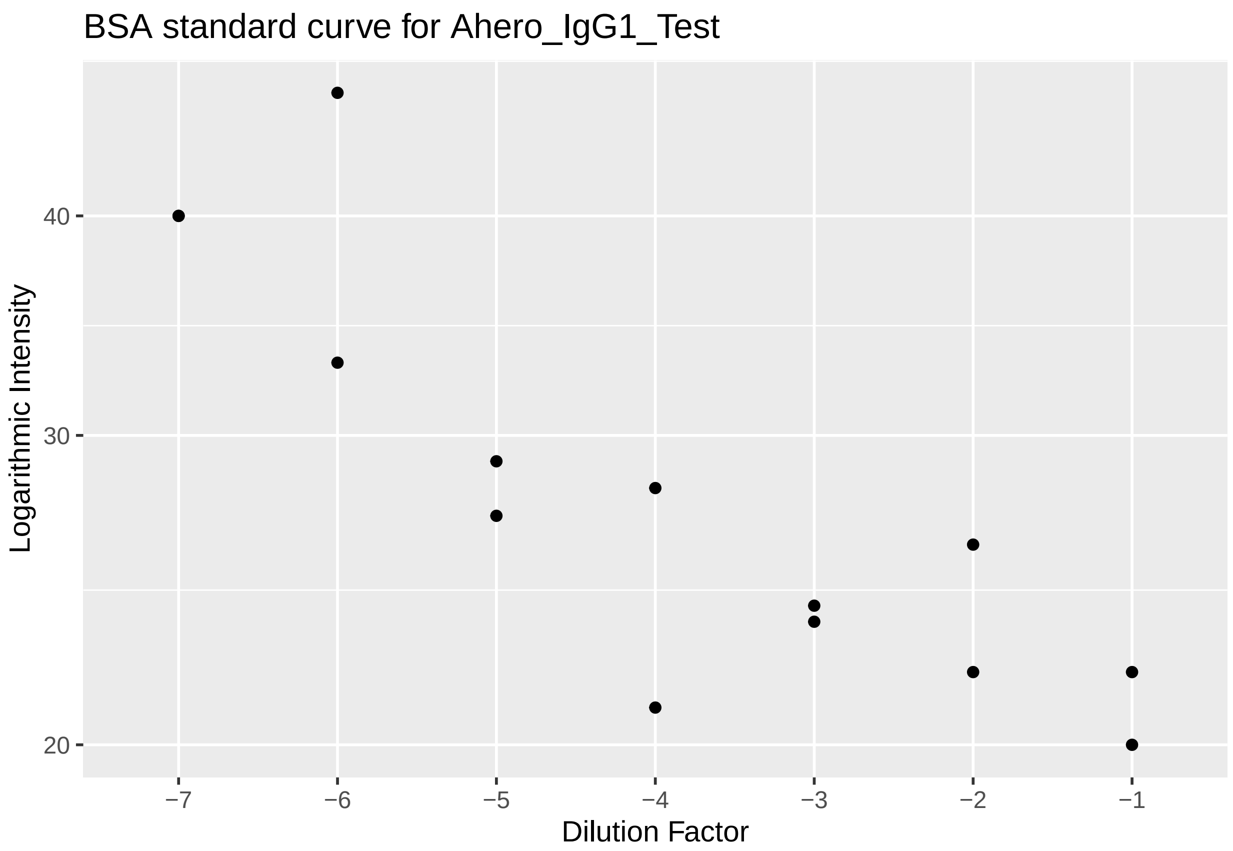
```
## Warning in self$trans$transform(x): NaNs produced
```

```
## Warning: Transformation introduced infinite values in continuous y-axis
```

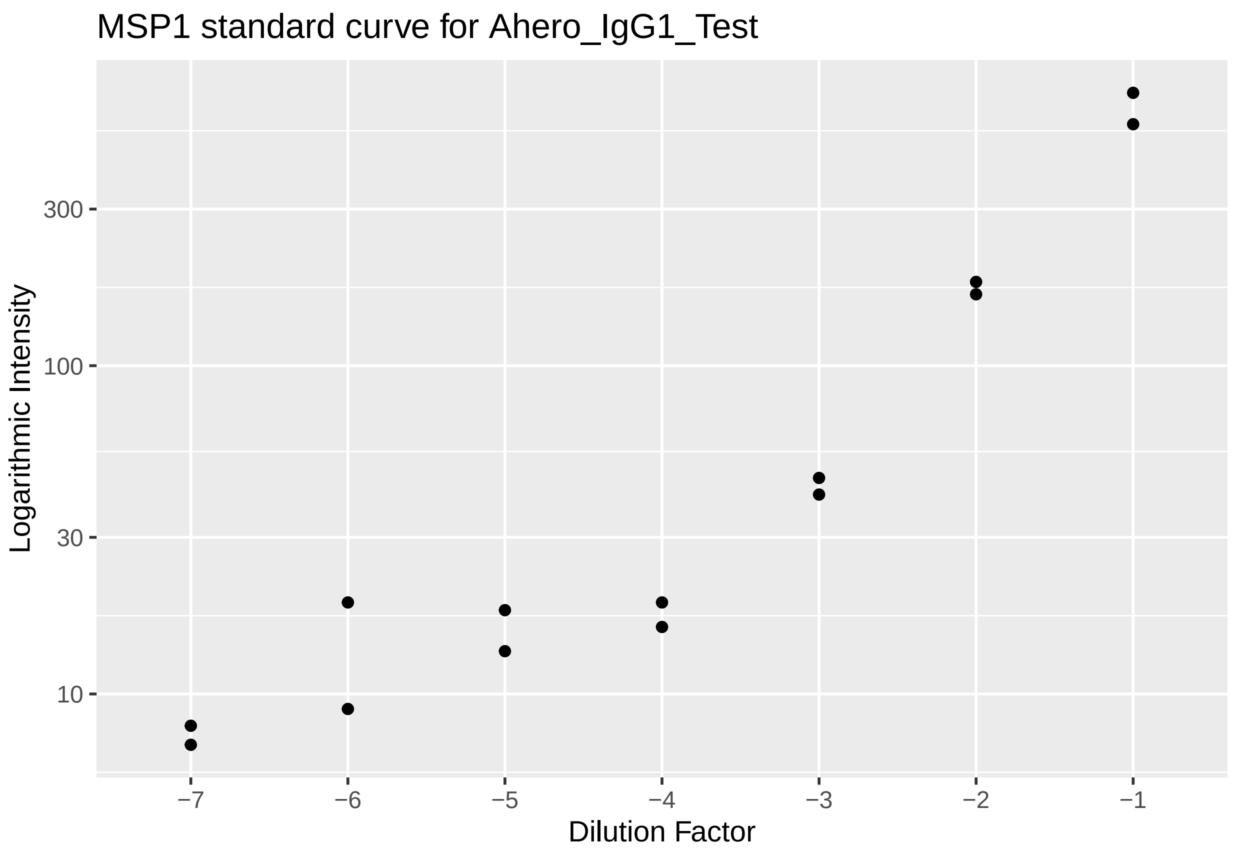
```
## Warning: Removed 1 rows containing missing values (‘geom_point()‘).
```


pp65 standard curve for Ahero_IgG1_Test
Logarithmic Intensity
10
3
1
Dilution Factor
−7
−6
−5
−4
−3
−2
−1

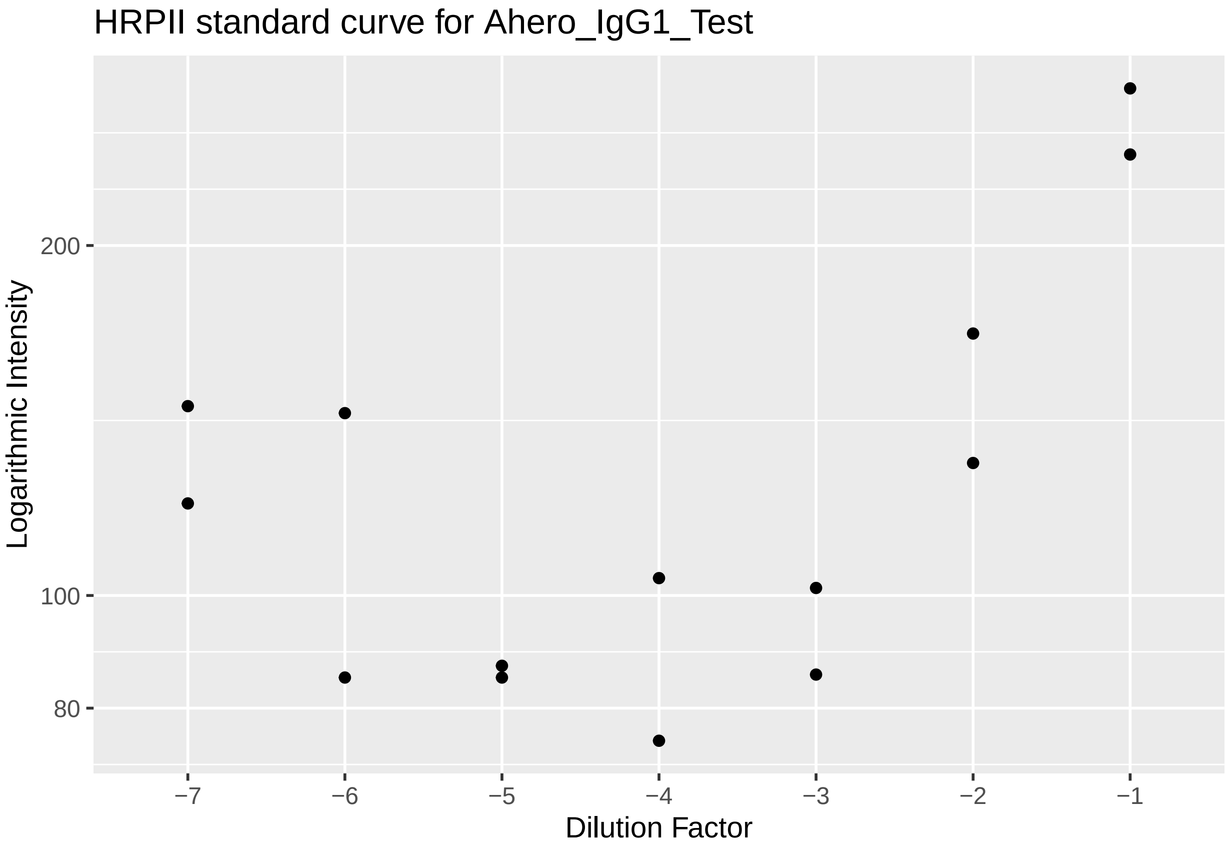
```
##
## [[5]]
```


BSA standard curve for Ahero_IgG1_Test
Logarithmic Intensity
40
30
20
−7
−6
−5
−4
−3
−2
−1
Dilution Factor

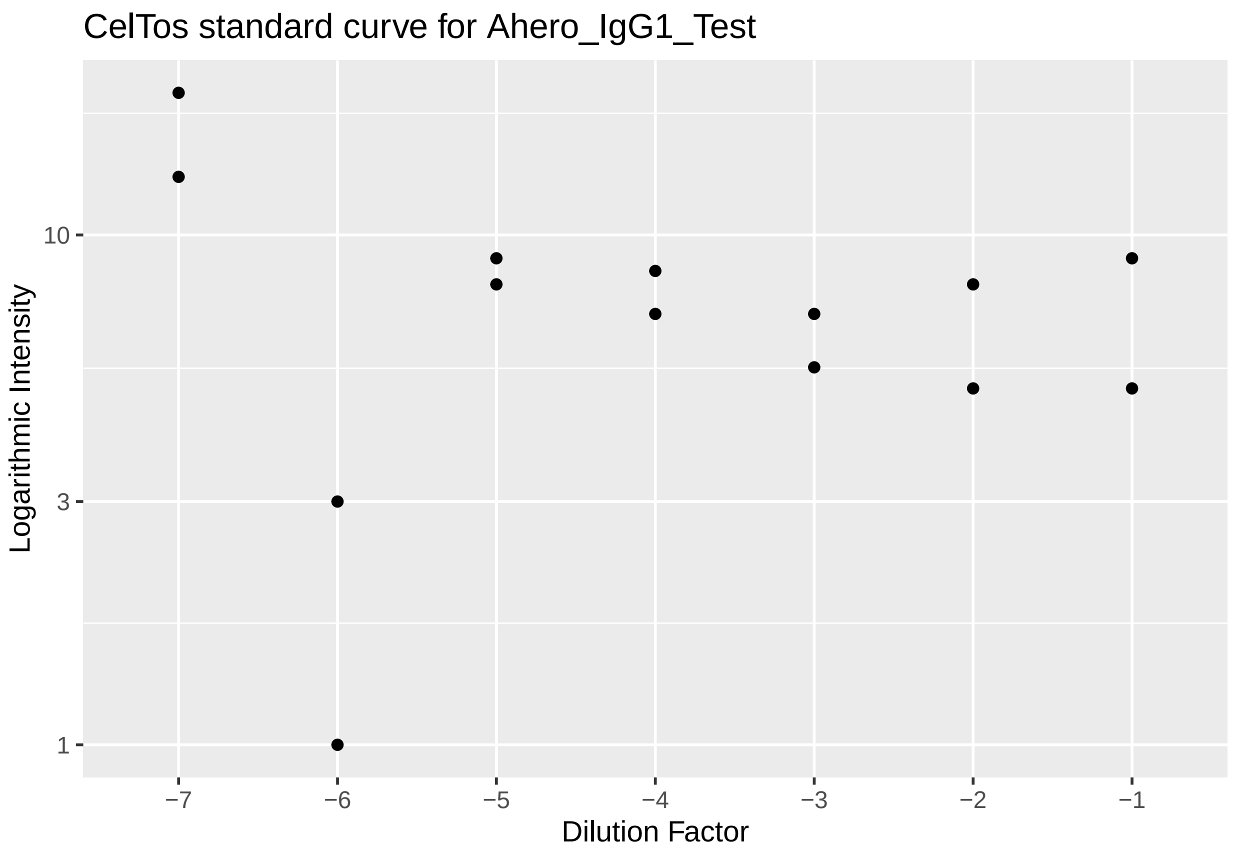
```
##
## [[6]]
```


MSP1 standard curve for Ahero_IgG1_Test
Logarithmic Intensity
300
100
30
10
−7
−6
−5
−4
−3
−2
−1
Dilution Factor

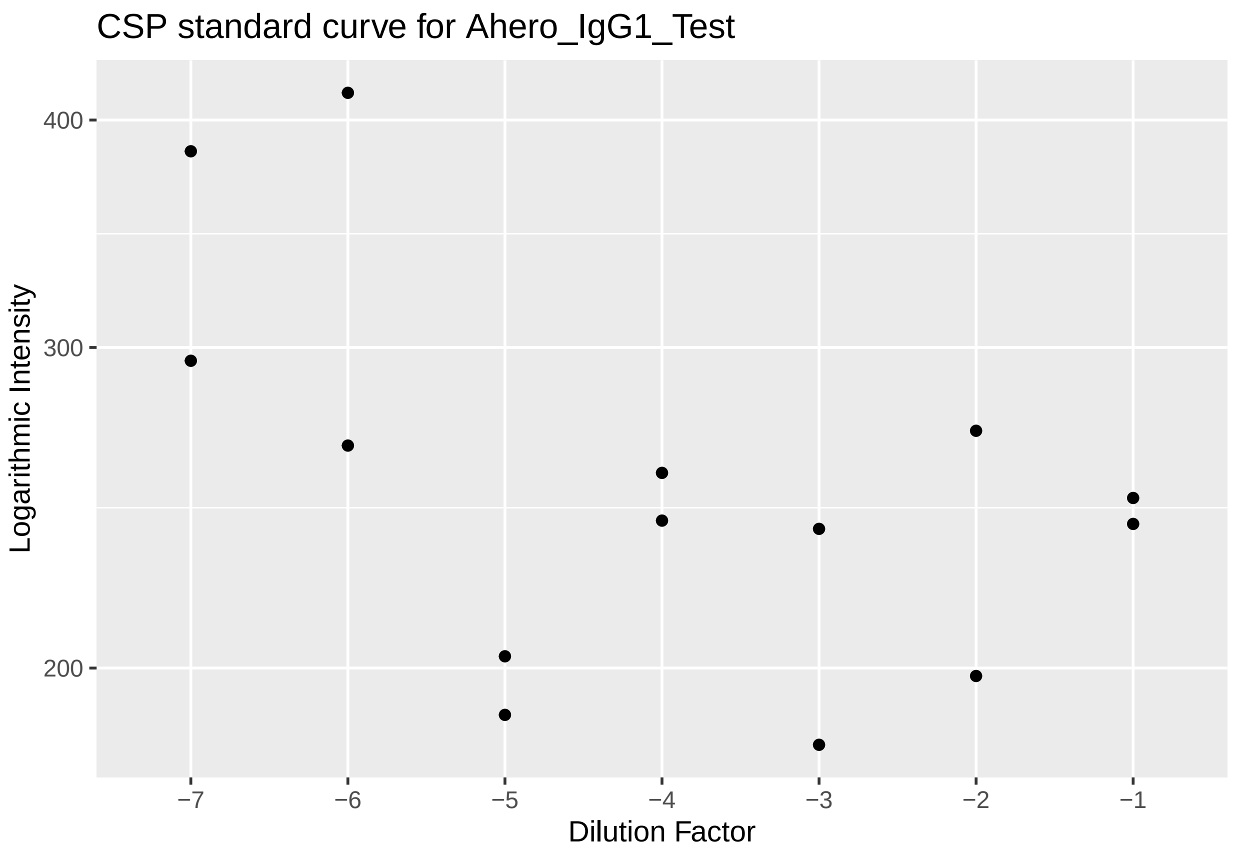
```
##
## [[7]]
```


HRPII standard curve for Ahero_IgG1_Test
Logarithmic Intensity
200
100
80
−7
−6
−5
−4
−3
−2
−1
Dilution Factor

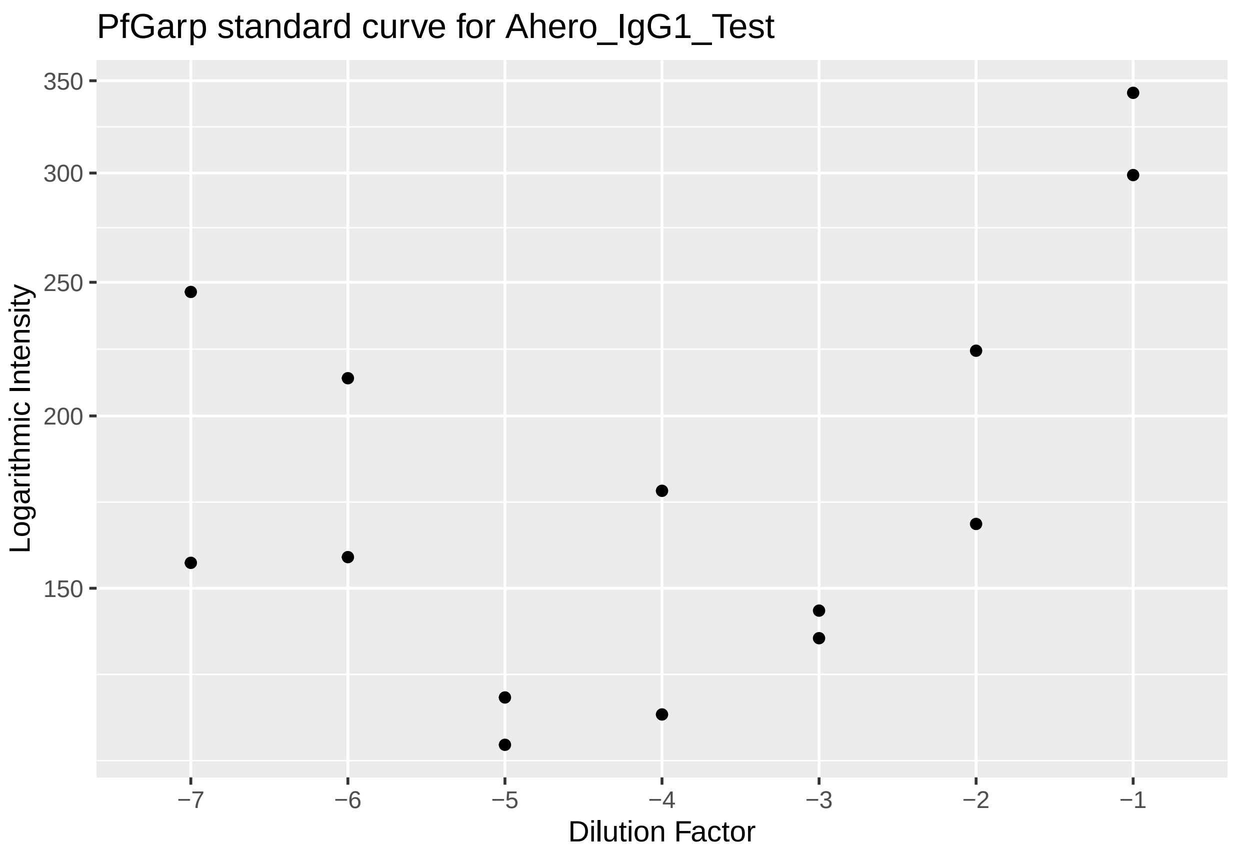
```
##
## [[8]]
```


CelTos standard curve for Ahero_IgG1_Test
Logarithmic Intensity
10
3
1
−7
−6
−5
−4
−3
−2
−1
Dilution Factor

```
##
## [[9]]
```


CSP standard curve for Ahero_IgG1_Test
Logarithmic Intensity
400
300
200
−7
−6
−5
−4
−3
−2
−1
Dilution Factor

```
##
## [[10]]
```


PfGarp standard curve for Ahero_IgG1_Test
Logarithmic Intensity
350
300
250
200
150
−7
−6
−5
−4
−3
−2
−1
Dilution Factor