**Assay setup and evaluation**

- TNF-alpha was used in a concentration of 1.66 ng/mL
- Humira (Adalimumab) titration by factor 2 was performed over 9 steps in triplicate starting from 0.32 µg/mL
- Humira was used with 100% and 80% potency level as sample
- AlamarBlue® fluorescence is shown on graph as response (linear)
- A 4-PL fit was used for potency determination
- Estimated vs stated potency: 80.3% vs 80%
- Assay was “passed” according to Eu. Pharm. Chapter 5.3

**Potency Estimation**

**80% Humira vs. 100% Humira**

- Potency Ratio (without Pre-Dilution Factors): 0.803
- 95.0% Confidence Interval: 0.736 – 0.876
- Relative Confidence Interval: 91.7% – 109.1% (17.4%)