AKRON BIO

TECHNICAL OVERVIEW
Specific & Biological Activity Reporting

Recombinant Human Interleukin-2 (rHu IL-2)

Lyophilized (10 µg) Cat. # AK8223-0010 | Lyophilized (100 µg) Cat. # AK8223-0100 | Liquid Syringe (1 mg) Cat. #AK9844-1000
Lyophilized (22 MIU) Cat. # AR1002-0022 | Lyophilized (1 mg) Cat. # AK8223-1000 | Liquid Bag (100 µg) Cat. #AR1045-0010

Variability in the Industry:

Ensuring the quality and consistency of ancillary materials used in the manufacture of cell and gene therapies is critical to both controlling costs and ensuring the integrity of the therapeutic product.

It is our responsibility as ancillary material manufacturers to reduce the variability that biomanufacturing presents. This is especially important when considering the manufacture of recombinant proteins for further manufacturing use. Because these products are biologics in and of themselves (in addition to the therapeutic products they enable), they represent critical materials that must be controlled and released in a way that ensures consistency. The primary means by which recombinant protein manufacturers can ensure consistency in therapy developers' manufacturing processes is by ensuring a reproducible process, as well as a reproducible means of measuring and reporting Specific and Biological Activity.

Activity Values:

Cell therapy companies often rely on the lot-specific Activity Values (reported in International Units) on their supplier's Certificates of Analysis (CoA) to measure the amount of material being used in their manufacturing processes. However, it is critical to realize that the values reported by different cGMP recombinant cytokine manufacturers cannot be directly compared with confidence if they do not adopt the same approach to measuring Activity and analyzing the results. As an industry, it is important to align around standard reporting backed by an accurate and reproducible consensus-based process for analyzing and reporting Activity Values. This limits variability and ultimately increases the consistency of the final therapeutic product.

Table 1:
Example of lot-specific activity values reported on CoA

TEST	RESULT
Specific Activity	14.38 MIU/mg
Biological Activity	13.23 MIU/vial

Specific activity: Expressed in IU/mg. Specific activity is inherent to the molecule and does not change, regardless of the concentration of the protein in the solution.

Biological activity: Expressed in IU/ml or IU/vial. Biological activity changes when the concentration of the protein in the solution changes.

Relative Potency and Parallel Line Assay:

To measure and report activity on Recombinant Human Interleukin-2 (rHu IL-2), Akron utilizes a parallel-line concentration-response model to estimate a relative potency compared to the National Institute for Biological Standards and Control (NIBSC 86/500) reference, applying the principles outlined in USP <1032> Design and Development of Biological Assays & USP <1034> Analysis of Biological Assays.

Relative Potency: Specific activity value (IU/mg) obtained for a test sample as a relative value compared to a reference standard's reported specific activity (IU/mg), by directly comparing the difference in concentration needed by the reference standard and the test sample to result in the same biological response for both.

Recombinant Human Interleukin-2 (rHu IL-2)

In comparison, other cGMP recombinant cytokine manufacturers take an alternative approach when calculating Activity Values. This, along with other differences in assay methodology, can result in reported values that are not directly comparable. We caution against using reported Activity Values to measure and dilute samples for comparability assays between different manufacturers. It is important for the industry to align with best practices around the determination and reporting of cytokine activity. Successfully identifying best practices and implementing standards can streamline the raw material qualification process, resulting in a more robust and resilient supply base, ensuring that therapy developers can assess suppliers accurately, and onboard secondary suppliers without the need for extensive and costly comparability assessments.

It is worth noting that the World Health Organization also chose to use a parallel-line approach when determining potency for the current International Standard for IL-2 (NIBSC 86/500).[1] Akron's reported rHu IL-2 Specific Activity levels are comparable to the Specific Activity of the current international standard (NIBSC 86/500), which is known to be approximately 13.73 MIU/mg.[2]

See below for a simplified example of the model Akron uses to compare relative potency by plotting biological response vs. concentration (log scale) for a test sample and a reference standard. The horizontal distance between these graphs represents the difference in concentration needed by the reference standard and the test sample to result in the same biological response for both. This defines relative potency.

Figure 1: Concentration-response model showing region applied in parallel line assay

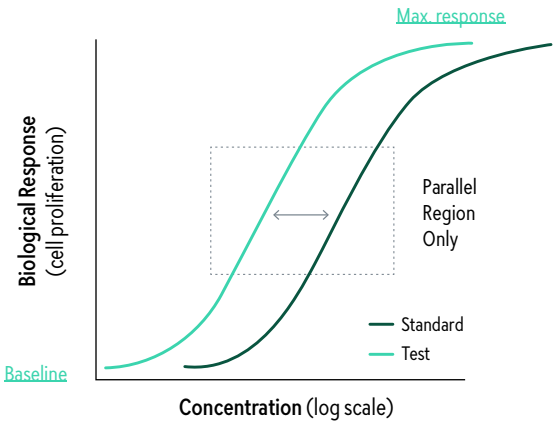
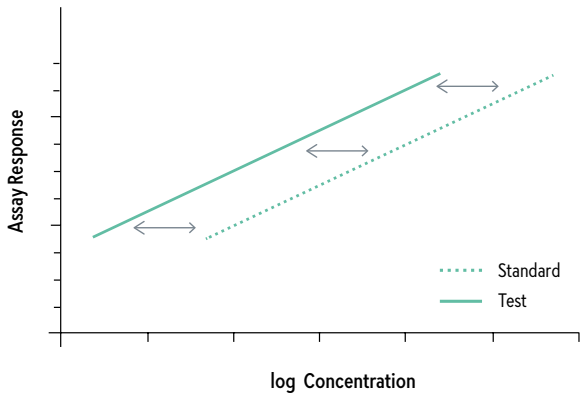


Figure 2: Relative potency displayed within linear portion of parallel line model per USP <1034> [3]



The parallel, linear regions for the test sample and reference standard in this model are compared to estimate a relative potency if–and only if–they first pass statistical validity tests for regression, linearity, and parallelism. Per USP <1032>, all biologically similar compounds will produce statistically similar results, but just because something has statistically similar results, does not ensure that it is biologically similar. Failure to produce statistically similar results, however, can be taken as evidence that they are not biologically similar.[4]

Table 2:
Tests of statistical validity for data points used in parallel line model

Test of Regresion (F-Test)		
$F_{regression}$	454.249	This test passes if Fregression > Fcritical
$F_{critical\ (95.0\%)}$	4.600	**Test Passed**
Test of Linearity (F-Test)		
$F_{non\text{-}linearity}$	1.065	This test passes if Fnon-linearity < Fcritical
$F_{critical\ (95.0\%)}$	3.112	**Test Passed**
Test of Parallelism (F-Test)		
$F_{non\text{-}parallelism}$	2.663	This test passes if Fnon-parallelism < Fcritical
$F_{critical\ (95.0\%)}$	4.600	**Test Passed**

Once found to be statistically valid, we assume biological similarity and parallelism, and a relative potency is calculated by comparing the concentration-response function of the test sample to the concentration-response function of the reference standard, as shown in the graph and equations below, adapted from USP <1034>.[3]

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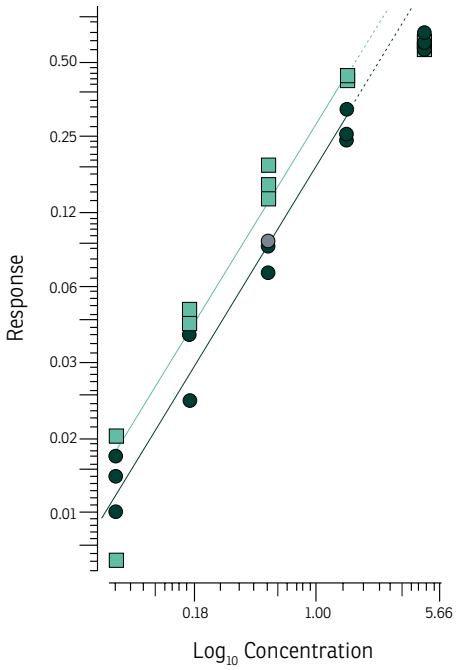
Figure 3: Relative potency determination using parallel line model via direct relationship between the test sample's and reference sample's concentration-response functions



FR(z) = concentration-response function for Reference (NIBSC)
FT(z) = concentration-response function for Test Sample
FT(z) = FR(ρz) ρ = relative potency z = concentration

NIBSC Standard FR(z) = αR + βlog(z)
FR(z) = αR + βx

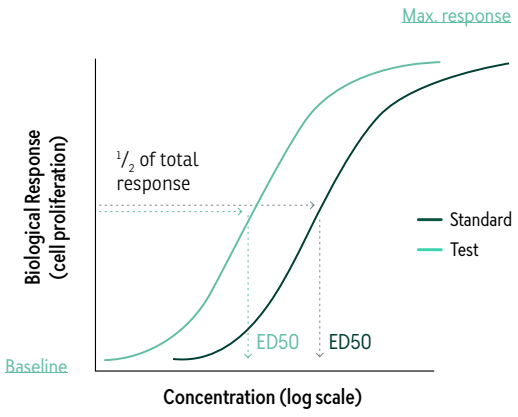
IL-2 test sample FT(z) = αR + βlog(ρz)
FT(z) = αR + βlog(ρ) + βlog(z)
FT(z) = αT + βx

If parallel: αT = αR + βlog(ρ)
relative potency **ρ = antilog [(αT - αR) / β]**

Alternative Method for Calculating Activity Levels:

The commonly employed alternative method for calculating Activity Levels, used by many cytokine manufacturers, is to determine the ED50 using the entire concentration-response graph and convert that directly into Specific Activity as shown in the graph and equation below.

Figure 4: Concentration-response model showing example ED50 comparison and equation used to convert ED50 value directly into specific activity (U/mg)



Specific activity (U/mg) = $1x10^6$ / ED50 (ng/mL)

ED50 is the concentration (ng/mL) that results in 50% of the maximum biological response. By applying the above equation, one can calculate a Specific Activity reported in U/mg. This then needs to be normalized against a reference with known potency so that Specific Activity in IU/mg can be reported, which is supposed to be a universally comparable value.

In our experience, for cytokine activity, there is more error involved when calculating a relative Specific Activity using the ED50 value rather than using a parallel-line model. This is supported by the following guidance in USP <1032> regarding variance:

"Simple analysis of quantitative bioassay data requires that the data be approximately normally distributed with near constant variance across the range of the data."[4]

"In practice, many bioassays have relatively large variation in log EC50 (compared to the variation in log relative potency) among assays (and sometimes among blocks within assay). If not addressed in the variance model, this variation in log EC50 induces what appears to be large variation in response near the mean log EC50..."[4]

Conclusion:

If manufacturers use different methods to measure and calculate Specific Activity, there will be completely different sources of error, resulting in Activity Values that should not be directly compared. Relative Activity Values can only be compared relatively, which means they must result from the same comparability testing protocol and data analysis methodology.

One of the simple options available for companies looking to compare cytokines from different manufacturers via comparability assays is to measure and dilute the test samples according to mass concentration, rather than the reported Specific Activity. If a consensus-based reference standard is included during this comparison, the relative Specific Activities of the test samples can be recalculated and compared using these results.

Akron has long been at the forefront of the efforts to coordinate and accelerate the development and diffusion of industry standards. It is in our interests, as a community of manufacturers seeking to accelerate the development and commercialization of cell and gene therapies, to standardize the assays that most directly impact our customers' manufacturing processes and ultimately the end patient experience. Only through greater standardization can we hope to achieve the resilient supply chains, cost of goods reductions, and manufacturing robustness that define a mature industry.

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References:

1) Wadhwa, M., Bird, C., Heath, A., Thorpe, R. Report on a Collaborative study for proposed 2nd International standard for Interleukin -2 (IL-2). Expert Committee on Biological Standardization WHO/BS/2012.2194
2) WHO International Standard 2nd International Standard for INTERLEUKIN 2 (Human, rDNA derived) NIBSC code: 86/500. Instructions for Use. (Version 1.0, Dated 23/04/2013)
3) USP <1034> Analysis of Biological Assays. USP-NF
4) USP <1032> Design and Development of Biological Assays. USP-NF