ELISA VALIDATION GUIDE

ASSAY FOR USE IN

DRUG DISCOVERY RESEARCH,
BIOPHARMA APPLICATIONS

KRISHGEN BioSystems

OUR REAGENTS, YOUR RESEARCH

VALIDATION OF KRIBIOLISA® ADUCANUMAB ELISA KIT (CATALOG NO. KBI1117) AS PER FDA/ICH GUIDELINES FOR BIOANALYTICAL METHOD VALIDATION

This validation protocol has been adopted in line with the Methodology and Analytical Procedures Guideline recommended by FDA/ICH.

Document History

First Codification	History	Date
Version#1	**VALIDATION DATA OF KRIBIOLISA® ADUCANUMAB ELISA (Cat No # KBI1117)**	30.11.2025

Approved Quality Control	Approved Product Development	Approved Operations Head
Prairna B	Atul G	K Jain

Introduction

This document presents a discussion of the characteristics of our **KRIBIOLISA® Aducanumab ELISA (Catalog No KBI1117)** kit considered by us during the validation of this kit in accordance with ICH Q2 (R1) guidelines. The document is prepared based on tests run in our laboratory and does not necessarily seek to cover the testing that may be required at user's end for registration in, or regulatory submissions. The objective of this validation is to demonstrate that it is suitable for its intended purpose - detection of **Aducanumab.**

Validation characteristics considered by us in accordance with the guidelines are listed below:

- **Assay Validation**
- **Standard Curve**
- **Pharmacokinetic Relevance**
- **Precision and Reproducibility**

The degree of revalidation required depends on the nature of the changes. Certain other changes may require validation as well.

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

For any queries or support on the data and its performance, please contact us at sales1@krishgen.com

Background

Aducanumab is a human IgG1 monoclonal antibody that selectively targets aggregated amyloid-beta (Aβ) plaques in the brain, promoting their clearance via microglia-mediated phagocytosis and reducing amyloid pathology associated with Alzheimer's disease. It is indicated for patients with mild cognitive impairment or mild dementia stage of the disease and is administered by intravenous infusion, with monitoring for amyloid-related imaging abnormalities (ARIA). Developed by Biogen in collaboration with Eisai, the drug received accelerated approval from the U.S. Food and Drug Administration on June 7, 2021, under the brand name Aduhelm based on its ability to reduce amyloid plaques, although its clinical benefit has been subject to ongoing debate.

1. Purpose

To assess the specificity, assay performance, and clinical relevance of the KRIBIOLISA® Aducanumab ELISA developed using Recombinant Human APP protease nexin 2 protein as capture protein.

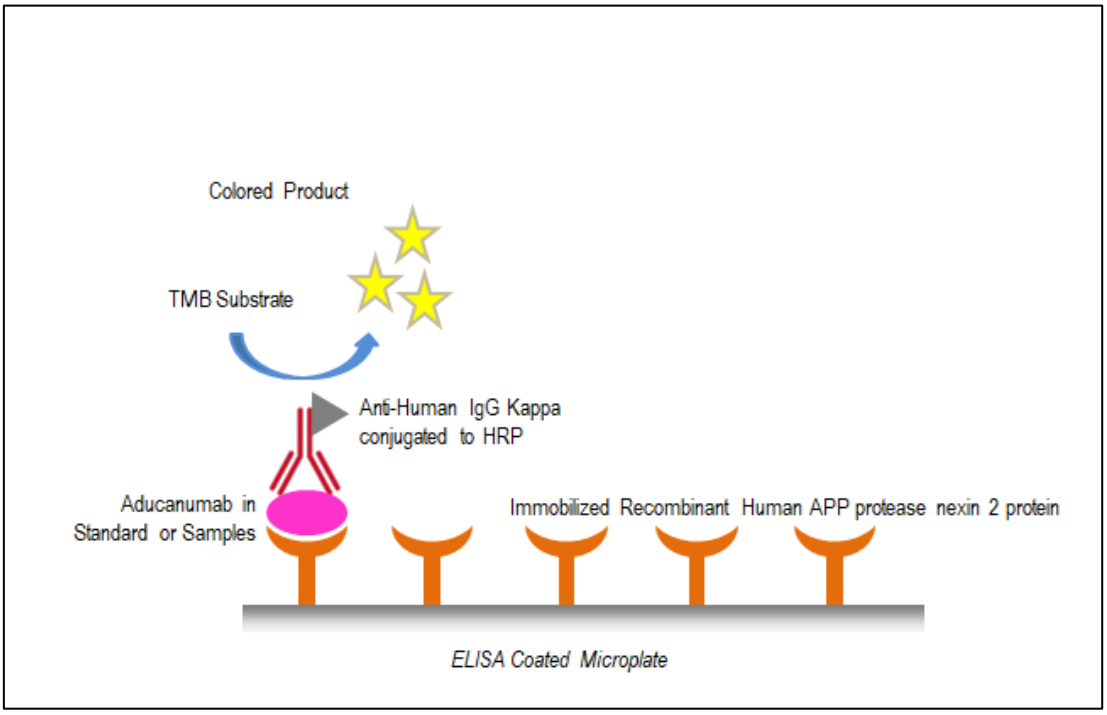
2. Experimental Design

- `A sandwich ELISA was performed using Human EGFR as capture protein.
- Standards prepared for Aducanumab.
- Assay Concentration Range: 0 - 500 ng/ml.

- Signal (% absorbance) plotted versus concentration.

The KRIBIOLISA Aducanumab ELISA employs a targeted immobilization strategy to ensure optimal presentation of recombinant human APP Protease Nexin 2 protein on the assay plate, thereby enhancing the selective binding of aducanumab. The immobilization process is carefully optimized to preserve the native conformation and relevant conformational epitopes of the protein, maintaining structural integrity and epitope accessibility. This controlled orientation ensures that the coated antigen is presented in a configuration that supports interaction with aducanumab's amyloid-beta–specific binding domains.

Aducanumab selectively recognizes conformational epitopes associated with amyloid-beta aggregates derived from the amyloid precursor protein (APP). Under these plate-bound conditions, this results in strong and stable antigen–antibody complex formation. In contrast, antibodies directed against unrelated APP regions or non-conformational epitopes may demonstrate reduced or limited binding. This differential binding behavior reflects the selective specificity of aducanumab as well as the preserved structural presentation of the immobilized recombinant APP Protease Nexin 2 protein established during the coating process.



ELISA kits for Aducanumab estimation offered by KRISHGEN uses Recombinant Human APP protease nexin 2 capture proteins as above

3. Assay Validation

- IC50 Value: ~ 7361 ng/ml (within 0-8000 ng/mL assay range).
- LLOQ: ~ 121.55 ng/ml.

- Clinical Cmax Values*:
  - After 10 mg/kg IV infusion (approved maintenance dose) :~ 180 – 200 µg/ml
  - After lower titration doses (1-6 mg/kg): dose –proportional Cmax values (~20 – 120 µg/mL depending on dose level)

- At steady state (after repeated monthly dosing) :~200–220 µg/mL

*Values are approximate and may vary depending on body weight, dosing schedule, and patient variability.

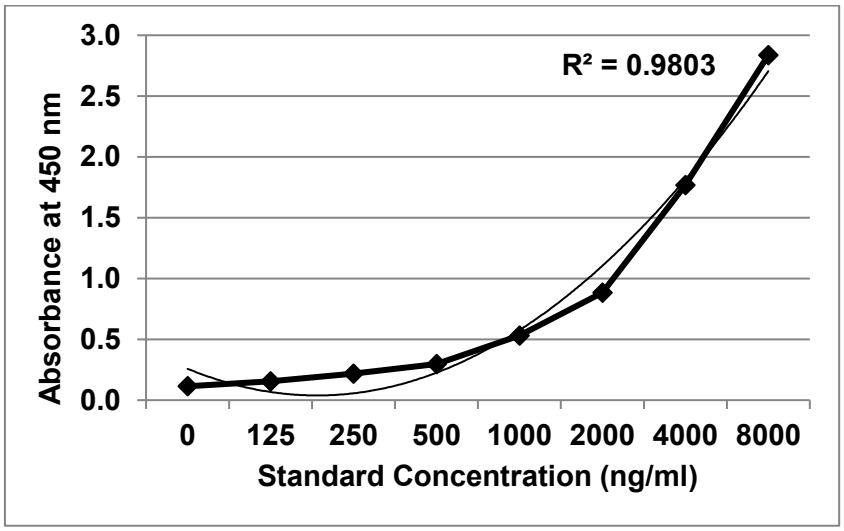
** published data*

- Precision:
  - Intra-Assay CV: <4%.
  - Inter-Assay CV: <8%.
  - Inter-Operator CV: <10%.

4. Standard Curve

Below is the standard curve for Aducanumab Sandwich ELISA assay:
Linearity and Range

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.114	--	--
125	0.156	109.9	87.9
250	0.218	299.8	119.9
500	0.297	519.4	103.9
1000	0.531	1079.4	107.9
2000	0.883	1874.0	93.7
4000	1.769	4079.5	102.0
8000	2.837	7972.5	99.7



5. LOD and LOQ

- LOD Absorbance: (Approx ~40.11 ng/ml)
- LOQ Absorbance: (Approx ~121.55 ng/ml)

6. Pharmacokinetic Relevance

The assay is designed to cover the clinically relevant serum concentrations of aducanumab observed following intravenous therapeutic dosing, making it suitable for pharmacokinetic evaluation and therapeutic monitoring. The Aducanumab ELISA demonstrates sensitivity within the µg/mL range, which lies well within the validated assay limits, ensuring accurate quantification across clinically meaningful exposure levels. Published pharmacokinetic data indicate systemic exposure consistent with therapeutic monoclonal antibodies used in Alzheimer's disease.

• Following the approved intravenous dosing regimen (10 mg/kg every four weeks after titration), the reported Cmax is approximately 180–200 µg/mL.
• With repeated monthly dosing, steady-state peak concentrations typically reach approximately 200–220 µg/mL.
• Exposure levels may vary depending on body weight, dose level during titration, infusion schedule, and individual patient variability.

Thus:

• At clinically relevant intravenous doses, aducanumab serum concentrations fall within the measurable range of the ELISA following appropriate sample dilution.
• The assay working range enables reliable differentiation across varying systemic exposure levels.
• Given the relatively high circulating concentrations associated with therapeutic dosing, routine dilution of clinical samples is recommended to ensure results fall within the linear dynamic range of the assay.
• The assay is therefore suitable for pharmacokinetic profiling, dose–exposure analysis, and therapeutic monitoring of aducanumab in human serum or plasma.

7. Precision and Reproducibility

Precision was assessed by analysing three standard concentrations (125 ng/ml, 2000 ng/ml, and 8000 ng/ml). Each concentration was tested in triplicate across three independent assay runs. %CV (Coefficient of Variation) was calculated within runs (intra-assay precision) and across runs (inter-assay precision).

Acceptance Criteria:

- Intra-assay %CV should be ≤15% for samples.
- Inter-assay %CV should be ≤15% for samples.
- %CV at LLOQ (Lower Limit of Quantitation) allowed up to 20%.

Precision Results Summary:

Standard (ng/ml)	Intra-Assay %CV (Range)	Inter-Assay %CV
125	3 % to 8%	<4%
2000	0.4 % to 4.2%	<7%
8000	0.1% to 1.4%	<2%

Observations:

- Intra-assay precision was consistently less than 8.5% across all levels tested.
- Inter-assay precision was consistently less than 7%.
- All precision values met the acceptance criteria for ELISA validation.

Conclusion:
The KRIBIOLISA® Aducanumab ELISA demonstrates excellent intra- and inter-assay precision. These results support the assay's reliability and reproducibility for routine use in pharmacokinetic and bioanalytical studies.

8. Conclusion

The KRIBIOLISA® Aducanumab ELISA is validated for sensitivity, specificity, precision, and accuracy, and is appropriate for pharmacokinetic applications in regulatory settings.

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