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Accusignal™ Nuclease ELISA Kit

An ELISA with broad sample compatibility and high sensitivity

Nuclease is an enzyme that plays a critical role in various aspects of pharmaceuticals, from drug discovery and development to gene therapy and quality control. It enables researchers and pharmaceutical companies to target specific genes and manipulate nucleic acids for therapeutic purposes.

The presence of nuclease must be addressed in the manufacture of therapeutic products, as it can degrade nucleic acids, whether the drug's genetic material or the patient's own genetic material. To meet quality and safety standards, it is essential to ensure the absence of impurities, such as nuclease. To address these concerns, pharmaceutical manufacturers may implement purification and quality control processes that may include steps to remove or inhibit nucleases, ensuring the final product is safe, stable, and effective for patients.

The Accusignal Nuclease ELISA Kit (KJE-4001) is designed to sensitively and robustly quantitate nuclease in a variety of therapeutic products and materials. This kit is a sandwich ELISA that uses pre-immobilized anti-nuclease antibodies and biotin-conjugated detection combined with streptavidin-HRP and 3,3',5,5'-tetramethylbenzidine (TMB) substrate to detect endonuclease from *Serratia marcencens*, such as Benzonase®, DENARASE®, and Turbonuclease.

Key Benefits

- **Sensitive antibody** for reliable detection of nuclease at low concentrations
- **Wide quantifiable range** with excellent dilution linearity for confidence in results across a large range of potential concentrations
- **Reproducible results** ensured by consistent low inter- and intra- plate variation
- **Antibody specificity** for use in materials that may use a variety of nuclease products from multiple manufacturers

Table A. General kit specifications

Specification	Parameter
LLOD	< 0.02 ng/mL
LLOQ	≤ 0.03 ng/mL
Range	0.03 – 20 ng/mL
Precision	< 20% intra- and inter- assay variability

A robust verification process was performed to prove the precision, accuracy, and repeatability of the Accusignal Nuclease ELISA Kit to assess nuclease concentrations in a variety of products at an acceptable range. The verification testing consisted of a minimum of triplicate replicates of each tested solution at each dilution over multiple days performed by multiple analysts.

The measured absorbance for each sample was interpolated using a four-parameter (4PL) logistic curve to calculate the sample concentration (in ng/mL).

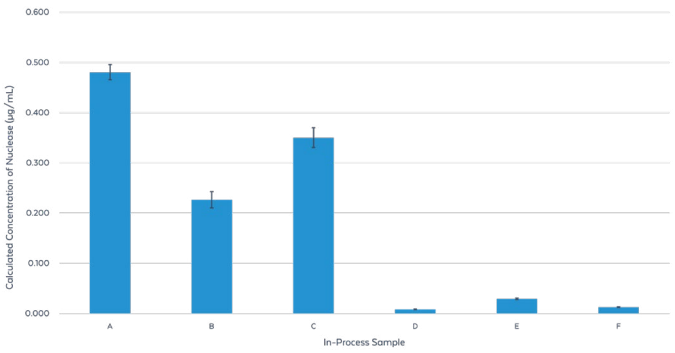

Fig. 1. Nuclease in in-process samples expressed as µg/mL. Error bars represent standard error.

Importance of Linearity

Dilution linearity across many in-process samples assesses the ability of the assay to provide accurate and linear measurements of a target analyte across a range of sample dilutions. This demonstrates sample compatibility and data confidence. As a sample is assessed across several dilutions,

the same calculated stock result must be observed, this is a parameter known as parallelism. The determination of acceptable parallelism indicates that the assay is reliable and can accurately quantify the target analyte in various sample types.

We have demonstrated sample compatibility and parallelism across several in-process samples.

The stock sample concentration (µg/mL) was interpolated from the mean absorbance values of multiple in-process samples across a range of dilutions using the 4-PL fit of the standard curve included with each kit.

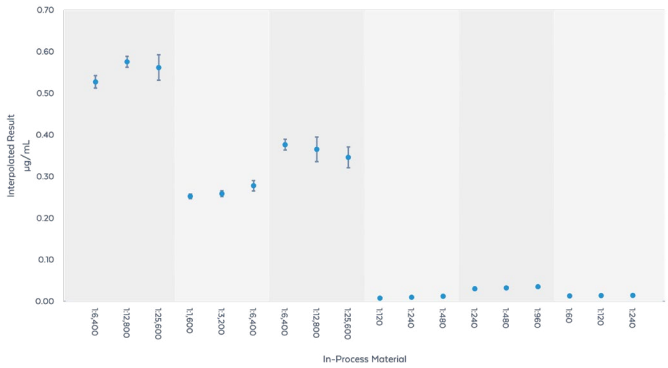

Fig. 2. Parallelism across in-process samples. Error bars represent standard error.

High Sensitivity

The Lower Limit of Quantitation (LLOQ) is the lowest concentration that can be reliably measured and with an acceptable degree of reproducibility.

The Lower Limit of Detection (LOD) is the lowest concentration that is distinguishable from the background.

To calculate the LOD and LLOQ, known concentrations of nuclease were spiked into sample buffer and measured using the kit. The LOD was calculated as the average background result plus three standard deviations thereof. The LLOQ was calculated as the lowest concentration for which recovery of 70-130% was observed with a coefficient of variation (CV) less than or equal to 25%. The LLOQ and LOD were verified across four independent assays.

Table 1. Lower Limit of Quantitation

Nuclease Concentration (ng/mL)	0.04	0.03	0.02
Mean Interpolated Concentration (ng/mL)	0.041 ± 0.001	0.032 ± 0.001	0.021 ± 0.001
Recovery (%)	104	106	104
CV (%)	13	23	44

Spike assays were utilized to confirm the LLOQ, testing a total of 84 replicate wells at each spike concentration level. At the 0.03 ng/mL spike concentration level, the 84 replicates resulted in an overall recovery of 106% and CV of 23%, confirming the LLOQ of 0.03 ng/mL.

In addition, 28 replicate wells of blank sample buffer were utilized to determine the LOD, resulting in a LOD with an OD of 0.0327, equivalent to 0.006 ng/mL.

Broad Dynamic Range

The AccuSignal Nuclease ELISA Kit has a broad dynamic range that reduces the number of plates and time needed for experiments.

We used the protein standard from the kit to prepare a standard curve from 20 to 0.03 ng/mL (Fig. 3, Table 2). The standard curve exhibits a broad range (0.03 to 20 ng/mL) and a strong goodness of fit (r2 ≥ 0.99).

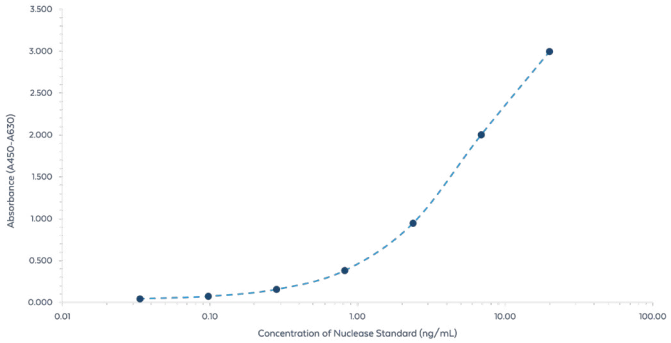

Fig. 3. Standard curve (4-PL fit) of the Nuclease Accusignal ELISA Kit.

Table 2. Mean absorbance and inter-plate CV of the Standard Curve

Concentration (ng/mL)	Mean Absorbance (A_{450} - A_{630})	Inter-Plate Variation (CV%)
20.00	2.998	6
6.90	2.004	7
2.38	0.948	9
0.82	0.383	8
0.28	0.158	6
0.10	0.074	6
0.03	0.045	5

To avoid assay repetition due to out-of-range detection, scientists need to determine the optimal dilution factors for each assay sample. For the best performance, we recommend performing not less than two dilution series in parallel. This combination will allow one to identify a dilution where the sample best generates a reading within the quantifiable range.

Robustness & Reproducibility

Assay precision is the Coefficient of Variation (CV) within a single experiment (intra-assay) and across multiple experiments (inter-assay). To perform precision studies, we spiked the assay with three sample concentrations (16, 2.25, and 0.15 ng/mL). We calculated intra-assay precision using four replicates for each concentration (Table 3) and calculated inter-assay precision from the averaged means of six replicate assays (six plates) (Table 4).

Table 3. Intra-Assay Precision

Spiked Nuclease Concentration (ng/mL)	Calculated Concentration (ng/mL)	Recovery (%)	CV (%)
16.0	15.6	98	3
2.25	2.32	103	2
0.15	0.14	95	3

Table 4. Inter-Assay Precision

Spiked Nuclease Concentration (ng/mL)	Calculated Concentration (ng/mL)	Recovery (%)	CV (%)
16.0	93	8	3
2.25	94	6	2
0.15	87	8	3

Flexibility & Compatibility

To test for possible matrix effects, we performed spike recovery assays with a panel of commonly used buffers. We spiked each matrix buffer with a concentration of nuclease, then performed and assayed a series of dilutions to assess dilutional linearity and parallelism.

For optimal performance, we recommend scientists should determine the optimal dilution factors for each assay sample. We also recommend routinely measuring the recovery of a spiked sample to detect any process-specific matrix effects. To do this, spike a known concentration of control protein into a control sample matrix.

Table 5. Matrix Recovery rates (%) demonstrate broad buffer compatibility

Matrix	Minimum Matrix Dilution Factor	Recovery (%)	CV (%)
50 mM Tris, pH 8.0	1	104	9
50 mM Sodium Phosphate, 0.3 M NaCl, 0.5 M Imidazole, pH 8.0	2	84	9
25 mM Sodium Phosphate, pH 7.5	1	103	16
50 mM Glycine, 50 mM Citric Acid, pH 2.0	24	94	6
25 mM Sodium Acetate, 0.5 M NaCl, pH 2.5	10	107	17
25 mM Citric Acid, 0.5 M NaCl, pH 2.0	10	89	14
100 mM Glycine, pH 3.5	10	94	7
50 mM Sodium Acetate, pH 3.2	10	97	8

Reagent Stability

We utilized an accelerated stability study to verify shelf life. We stored kit components at 25°C and tested assay performance at regular intervals over 98 days using the criteria in Table 6. An accelerated study at 25°C over 98 days is equivalent to 12 months at the intended storage condition of 2-8°C according to the Variable Q10 Method.

All components met acceptance criteria during accelerated stress testing at day 98. This indicates that the predicted shelf life of the kit is 12 months from the manufacture date when stored at 2-8°C.

Table 6. Test Criteria and results of accelerated stability study

Parameter	Pass Criteria	Result (Day 98 at 25°C)
Absorbance of 6.9 ng/mL Standard	> 1.0	Pass
Absorbance of Sample Buffer	< 0.1	Pass
Intra-Assay CV	< 20%	Pass
LLOD (ng/mL)	< 0.03	Pass
Recovery of each Protein Standard	80-120%	Pass

Kit Components

Table B. List of Accusignal Nuclease ELISA Kit Components

Component	Item No.	Size
Nuclease Antibody-coated 96-well Strip Plate	KJE-4001B	1 plate
Biotinylated Anti-Nuclease Detection Antibody (100X)	KJE-4601A	110 µL
Nuclease Protein Standard	KJE-0001C	0.2 µg
Streptavidin-HRP (100X)	KJX-0001K	110 µL
Sample Buffer	KJX-0001D	50 mL
Stop Buffer	KJX-0001G	20 mL
TMB Buffer	KJX-0001F	20 mL
Wash Buffer (10X)	KJX-0001E	60 mL
Plate Sealer	KJX-0001H	1 sheet

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