R&D systems by bio-techne

Endotoxin Kinetic Quantitation Kit

Kinetic Chromogenic Assay

Catalog Numbers: EKC032 (32 test)

EKC064 (64 tests)

PRODUCT DESCRIPTION

Kinetic Chromogenic LAL Endotoxin Quantitation assays are widely used for the *in vitro* detection and quantification of endotoxins, specifically lipopolysaccharides (LPS), which are key structural components of the outer membrane of Gram-negative bacteria. During bacterial growth and especially upon cell lysis, these highly pyrogenic molecules are released into the environment, where they can trigger strong inflammatory and immune responses in mammalian systems, even at very low concentrations. Because endotoxins are extremely heat-stable and resistant to conventional sterilization methods, contamination can occur through water, raw materials, laboratory equipment, containers, or inadequate aseptic practices, presenting significant risks to product safety and quality.

The R&D Systems™ Endotoxin Kinetic Chromogenic Quantitation Kit offers continuous, real-time monitoring of endotoxin-activated enzymatic reactions. This capability enables precise, sensitive, and reliable quantification, supporting stringent endotoxin control in pharmaceuticals, biologics, and laboratory products, and workflows. It provides improved accuracy for samples with variable turbidity or slow-reacting matrices and is particularly suited for complex sample types, applications requiring compliance-level quantitation, and workflows that demand low-level endotoxin detection with high reproducibility.

The kit includes a Lyophilized Amebocyte Lysate (AL) Reagent, sourced from the circulating amebocytes of the Asian horseshoe crab, *Tachypleus tridentatus*. In the presence of endotoxins, proenzymes in the lysate are activated to form coagulase enzymes, which catalyze the hydrolysis of a colorless substrate, releasing the yellow chromophore p-nitroaniline (pNA). The resulting color intensity is measured quantitatively at 405 nm, and the rate of color formation is directly correlated with the endotoxin concentration. A fast absorbance increase indicates a high endotoxin concentration, whereas a slow increase indicates a low concentration. Endotoxin levels are determined by comparing the sample reaction rate to a standard curve.

INTENDED USE

For Research Use Only. Not for use in diagnostic procedures.

R&D Systems Endotoxin Kinetic Quantitation Kit is designed for *in vitro* quantitative detection of Gram-negative bacterial endotoxins (Lipopolysaccharides) using a kinetic chromogenic method.

MATERIALS REQUIRED *(NOT PROVIDED)*

- Pyrogen-free pipettes, 1 mL, 100 µL, or automatic pipettes with pyrogen-free tips
- Pyrogen-free 96-well microplates or plate strips
- Test tube rack
- Incubating microplate reader equipped with a 405 nm filter and kinetic assay software
- Pyrogen-free reagent reservoirs (optional)
- Multi-channel pipettor (optional)
- Vortex mixer
- Timer

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MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

COMPONENT	KIT CATALOG #	QUANTITY PER TEST KIT	CAP COLOR	DESCRIPTION	STORAGE OF MATERIAL
Chromogenic Amebocyte Lysate (AL)	EKC032	2 vials (1.7 mL/vial)	Blue	Lyophilized Reagent containing Amebocyte Lysate (AL) and chromogenic substrate. Detection limit: 0.005 EU/mL.	Store at 2-8 °C. Use within 10 minutes of reconstitution. **Protect from light.**
	EKC064	4 vials (1.7 mL/vial)			
Reconstitution Buffer	EKC032	2 vials (3 mL/vial)	Silver	Buffer for rehydrating Chromogenic AL Reagent.	Store at 2-8 °C. Bring to room temperature before use.
	EKC064	4 vials (3 mL/vial)			
Endotoxin Control Standard	EKC032	2 vials	Red	Lyophilized Endotoxin Standard (*E. coli* 0111:B4). *Potency on Certificate of Analysis.*	Store at 2-8 °C. Reconstituted solution stable one week at 2-8 °C. **Do not freeze.**
	EKC064	4 vials			
Endotoxin-free Water	EKC032	1 x 50 mL bottle	Blue	Endotoxin-free Water (< 0.005 EU/mL).	Store at 2-30 °C.
	EKC064	2 x 50 mL bottle			

SAMPLE COLLECTION AND PREPARATION

All glassware, plasticware, and diluents contacting samples or reagents must be endotoxin-free. Glassware and other heat-stable apparatus can be depyrogenated in oven using a validated process, a commonly used minimum time and temperature setting is 60 minutes at 250 °C. Store samples under conditions that prevent bacteriological activity. For temporary storage (less than 24 hours), keep samples at 2-8 °C; for long-term storage, keep samples below -10 °C.

The optimal pH range for the Amebocyte Lysate-Endotoxin reaction is 6-8. Adjust acidic or basic samples to this range using endotoxin-free 0.1 N sodium hydroxide, 0.1 N hydrochloric acid, or endotoxin-free Tris buffer. Always measure the pH of an aliquot of the bulk sample to avoid contaminating the main sample with the pH electrode. Test for and eliminate potential interfering substances as described in the Product Inhibition/Enhancement section.

REAGENT PREPARATION

Allow reagents to reach room temperature before use. Prepare all solutions at room temperature.

Preparation of Endotoxin Standards

Note: *Reconstitute Endotoxin Control Standards immediately before use.*

1. Reconstitute Endotoxin Control Standard with Endotoxin-free Water using the volume specified in the Certificate of Analysis. Vortex vigorously for 5 minutes to obtain a 50 EU/mL Endotoxin Stock Solution. The reconstituted stock is stable for 7 days at 2-8 °C. **Do not freeze.**
2. Preparation of Endotoxin Standard range 0.005 - 5 EU/mL

CONCENTRATION	INSTRUCTIONS
5 EU/mL	Mix 0.1 mL of 50 EU/mL Endotoxin Stock Solution with 0.9 mL Endotoxin-free Water, vortex for 1 minute to obtain a 5 EU/mL endotoxin solution.
0.5 EU/mL	Mix 0.1 mL of 5 EU/mL endotoxin solution with 0.9 mL Endotoxin-free Water, vortex for 1 minute to obtain a 0.5 EU/mL endotoxin solution.
0.05 EU/mL	Mix 0.1 mL of 0.5 EU/mL endotoxin solution with 0.9 mL Endotoxin-free Water, vortex for 1 minute to obtain a 0.05 EU/mL endotoxin solution.
0.005 EU/mL	Mix 0.1 mL of 0.05 EU/mL endotoxin solution with 0.9 mL Endotoxin-free Water, vortex for 1 minute to obtain a 0.005 EU/mL endotoxin solution.
Blank	Use 0.5 mL of Endotoxin-free Water.

3. Discard the remaining endotoxin dilutions

Preparation of AL Reagent:

1. Reconstitute each vial of Chromogenic AL Reagent with labeled amount of Reconstitution Buffer.
2. Mix gently by tilting and swirling the vial until the contents are in solution.
   Note: *Do not use vortex mixer. Reconstituted Chromogenic AL reagent solution should be used within 10 minutes at room temperature. If more than one vial is required, pool two or more vials before use.*

SETTING MICROPLATE READER

1. Set the incubator temperature at 37 ºC.
2. Set up the template and procedure.
3. Set wavelength at 405 nm and onset OD at 0.2.
4. Set the reading parameters as kinetic reading for 120 minutes with interval of 60 seconds.
5. Set the plate shaking speed as medium speed for 5 seconds before the kinetic reading starts.

TEST PROCEDURE

1. Run the assay after the kinetic incubator temperature reached 37 °C.
2. Run assay in duplicate.
3. Vortex endotoxin standards and samples before loading.
4. Transfer 100 µL of Endotoxin-free Water (as negative control), endotoxin standard solutions, and test sample into each well of the microplate. Avoid air bubbles.
5. Transfer reconstituted Chromogenic AL Reagents into a reagent reservoir, mix by rocking the reservoir from side to side. Add 100 µL Chromogenic AL Reagent into each well of the microplate rapidly. Application of multiple-channel pipettor or repeating pipettor is recommended. Avoid air bubbles. Leave the microplate uncovered.
6. Place the microplate into the kinetic microplate reader.
7. Run the kinetic program. The program should include a plate-shaking step at medium speed for 5 seconds before the kinetic run begins to ensure thorough mixing of the Chromogenic AL Reagent and the test samples. Record the kinetic readings every 60 seconds for 120 minutes at wavelength of 405 nm.

DATA COLLECTION AND ANALYSIS

1. Obtain the onset time for onset O.D 0.2 at 405 nm by Certificate of Analysis.
2. Construct the Standard Curve

   log 10 y = a (log 10 x) + b, where

   y = reaction time (onset time), x = endotoxin concentration,

   a = slope of the regression curve, b = the Y intercept.

ROUTINE TESTING

For routine testing, prepare a series of endotoxin standards and assay them alongside the unknown samples under the same conditions. Determine the endotoxin concentration in the unknowns by comparing performance to the standards. Each standard and unknown sample should be tested in at least duplicate, and the average absorbance of all replicates should be used for calculations. A Positive Product Control (PPC) may be included to monitor for product inhibition or enhancement.

INITIAL QUALIFICATION

As recommended in the Pharmacopeia, validation of the Chromogenic Amebocyte Lysate Endotoxin assay should be performed when conditions are likely to influence the test result change.

Standard Curve Validation. Upon receipt of a new lot of Chromogenic Amebocyte Lysate (AL) reagent, a standard curve validation test must be performed. Prepare at least three endotoxin concentrations within the standard curve range specified in the Certificate of Analysis. The selected concentrations should bracket each log increase within the range (e.g., 0.005, 0.05, 0.5, and 5 EU/mL for a 0.005-5 EU/mL range). Perform the assay using at least three replicates for each endotoxin concentration. Unlike routine testing, do not average the onset times of all replicates. The absolute correlation coefficient (r) must be ≥ 0.980 for the tested endotoxin range.

Test for Interfering Factors. The test for interfering factors must be repeated whenever conditions that could affect test results change. This includes, but is not limited to, modifications to sample formulation or use of a new Chromogenic AL reagent supplier. Refer to the Product Inhibition/Enhancement section for guidance on identifying and mitigating potential interference.

PERFORMANCE CHARACTERISTICS

The test is valid only when all of the following requirements are met.

1. Prepare at least three endotoxin concentrations within the specified range, along with blanks, to generate the standard curve. Perform the assay using at least two replicates for each endotoxin concentration.
2. The absolute value of the correlation coefficient (r) of the calculated standard curve should be ≥ 0.980.
3. The onset times of both negative controls must be longer than those of the lowest endotoxin standard concentration.

PRODUCT INHIBITION/ENHANCEMENT

If there is potential that the sample contains interfering substances, recovery rate test should be run. Prepare a Positive Product Control (PPC), which is a sample of the product spiked with a known amount of endotoxin. The spike concentration of endotoxin (λm) should be in the middle of the standard curve range.

1. Analyze the spiked sample (PPC) along with the unspiked sample.
2. Determine the endotoxin concentration in spiked test sample (Cs) and in unspiked test sample (Ct).
3. Calculate the recovery rate (R): $R = (Cs-Ct) / \lambda m \times 100\%$.
4. Recovery rates within the range of 50-200% suggest non-significant interference. Recovery rates out of the range of 50-200% suggest significant interference. Dilution and modification are commonly employed to reduce the interference to a non-significant level.
5. If R is outside the 50-200% range, repeat the inhibition test using a series of test sample dilutions, ensuring that the dilution does not exceed the Maximum Valid Dilution.
6. For routine endotoxin concentration assays, it is best to select a test sample dilution at which R is closest to 100%.

COLORED SAMPLES

For colored samples, if the absorbance of the colored sample is greater than 1.5 O.D the sample should be diluted in Endotoxin-free Water until the absorbance is less than 1.5 O.D.

REFERENCES

- FDA (1987) Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test.
- USP (2023) Bacterial Endotoxins Test. United States Pharmacopeia <85>.
- Bang, F.B. (1956) Bull. Johns Hopkins Hosp. 98:325-351.
- Levin, J. and Bang, F.B. (1964) Bull. Johns Hopkins Hosp. 115:337-345.
- Levin, J. and Bang, F.B. (1964) Bull. Johns Hopkins Hosp. 115:265-274.
- Levin, J. and Bang, F.B. (1968) Thromb. Diath. Haemorrh. 19:186-197.