Version 2 Last updated 9 May 2023

ab273269
3' to 5' Exonuclease Activity Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273269
(use https://www.abcam.cn/ab273269 for china, or
https://www.abcam.co.jp/ab273269 for Japan)

For the determination of 3' to 5' Exonuclease activity in a variety of biological samples.

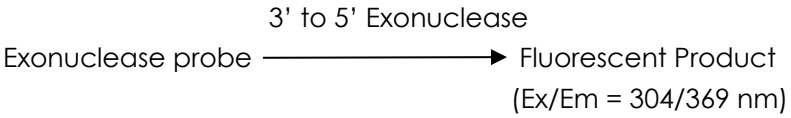
This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

3' to 5' Exonuclease Activity Assay Kit (Fluorometric) (ab273269) provides a quick and easy method for monitoring 3' exonuclease activities in a wide variety of samples. In this assay, exonuclease digests the provided DNA probe, producing a strong fluorescent signal (Ex/Em = 304/369nm). The kit is simple, sensitive, high-throughput adaptable and can detect as low as 0.2 µU of exonuclease activity.



2. Protocol Summary

Prepare lysates as directed and measure protein concentration

Prepare all reagents as directed

Add Positive Control, Samples and Background Control to appropriate wells

Add Sample Reaction Mix (to Samples and Positive Control) and Background Reaction Mix (Background Control) to appropriate wells

Measure fluorescence (Ex/Em = 304/369 nm) in kinetic mode for 30 - 60 minutes at room temperature.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
Exonuclease Assay Buffer	25 mL	-20°C
Exonuclease Probe	1 vial	-20°C
96-Well Half Area Plate (with lid)/Half-area Plate	1 unit	-20°C
Fluorescence Standard II/Fluorescence Standard (2 mM in DMSO)	100 µL	-20°C
Exonuclease Positive Control	1 vial	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Spectrophotometer
- Dounce Homogenizer
- Sonicator (for bacterial culture)
- 100% saturated Ammonium Sulfate solution (for animal tissue)
- Molecular Biology Grade Water

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Exonuclease Assay Buffer

Ready to use. Bring to room temperature before use. Store at RT.

9.2 Exonuclease Probe:

Reconstitute with 110 µL Molecular Biology Grade Water. Aliquot and store at -20ºC. Avoid multiple freeze-thaw cycles. Keep away from light.

9.3 96-Well Half Area Plate (with lid)/Half-area Plate:

Ready to use. Store at RT.

9.4 Fluorescence Standard II/Fluorescence Standard (2 mM in DMSO):

Ready to use. Warm to RT. Store at -20°C.

9.5 Exonuclease Positive Control

Reconstitute with 100 µL Exonuclease Assay Buffer. Aliquot and store at -20ºC. Avoid multiple freeze-thaw cycles.

10. Sample Preparation

10.1 Bacterial cell culture:

10.1.1 Collect bacterial cells from 1 ml culture by centrifugation at 4000 x *g* for 10 min.

10.1.2 Discard the supernatant and resuspend with 100 µL Exonuclease Assay Buffer. Lyse the bacterial cells with a sonicator.

10.1.3 Centrifuge the lysate at >10,000 x g and 4°C for 10 min and collect the supernatant.

10.2 Mammalian cell culture:

10.2.1 Collect 500,000-1,000,000 mammalian cells by centrifugation.

10.2.2 Resuspend the cell pellet in 100 µL Exonuclease Assay Buffer and rapidly homogenize the solution with a Dounce homogenizer.

10.2.3 Centrifuge the lysate at >10,000 x *g* and 4°C for 10 min and collect the supernatant.

10.3 Animal tissue:

10.3.1 Rapidly homogenize 10 µg of tissue in 100 µL Exonulcease Assay Buffer with a Dounce homogenizer.

10.3.2 Centrifuge the lysate at >10,000 x *g* and 4°C for 10 min and collect the supernatant.

10.3.3 Add 2 volumes of saturated ammonium sulfate solution into the supernatant (200 µL $(NH4)_2SO_4$ for 100 µL of Sample). Place on ice for 30 min to precipitate the protein.

10.3.4 Centrifuge at >10,000 x *g* and 4°C for 10 min and collect the precipitate.

10.3.5 Resuspend the precipitated protein in 100 µL Exonuclease Assay Buffer. Pipette up and down to make sure that the precipitated protein completely dissolves.

Δ Note: Exonuclease Buffer contains DTT. A reducing agent-compatible BCA protein assay kit should be used for Sample protein concentration measurement if you wish to express activity as µU/µg protein.

11. Standard Curve

11.1 Make 100 µM Standard solution by adding 50 µL of the 2 mM Fluorescence Standard II/Fluorescence Standard into 950 µl of dH_2O.

11.2 Make a 5 µM Standard solution by adding 10 µL of 100 µM Standard solution into 190 µL of dH_2O.

11.3 Add 0, 2, 4, 6, 8, 10 µL of the 5 µM Standard solution into a series of wells in the 96-Well Half Area Plate (with lid)/half-area plate resulting in 0, 10, 20, 30, 40, 50 pmol of Standard/well.

11.4 Adjust the volume to 50 µL/well with Exonuclease Assay Buffer.

Standard #	5 µM Standard (µL)	Exonuclease Assay Buffer (µL)	Standard (pmol/well)
1	10	40	50
2	8	42	40
3	6	44	30
4	4	46	20
5	2	48	10
6	0	50	0

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

12.1.1 Mix enough reagents for the number of assays (Samples, Sample Background Control & Positive Control) to be performed. For each well, prepare 25 µL Reaction Mix containing:

Reaction mixes:

12.1.2 Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µl of the Substrate Mix:

Component	Sample Reaction Mix	Background Reaction Mix
Exonuclease Probe	1 µL	-
Molecular Biology Grade H_2O	24 µL	25 µL

12.1.3 **Standard Curve:** Add 50 µL of each standard to appropriate wells ready for reading.

12.1.4 **All Samples: :** Add 2-25 µL of the Sample in two parallel wells in the 96-Well Half Area Plate (with lid)/Half-area plate and label as Sample (S) and Sample Background Control (BC). Make up all Sample, Sample Background Control and Positive Control wells to 25 µL with Exonuclease Assay Buffer. Prepare several dilutions to make sure that the kinetic curve falls in the range of the Standard Curve.

12.1.5 **Positive Control:** Add 5-10 µL of the Exonuclease Positive Control into a well in the 96-Well Half Area Plate (with lid)/Half-area Plate.

12.1.6 Mix and add 25 µL of the Sample Reaction Mix to each well containing the Samples (S) and Positive Control.

12.1.7 Add 25 µL of the Background Reaction Mix into the wells containing Sample Background Control (BC).

12.1.8 Mix wells for 30-60 seconds.

12.1.9 Measure the fluorescence (Ex/Em = 304/369 nm) in kinetic mode every 30 seconds for 30-60 min at 37°C.

Δ **Note:** Adjust GAIN/PMT setting of your fluorometer as necessary so that the Standard Curve readings are linear within the detection range of the instrument.

13. Calculations

13.1 Subtract 0 pmol Standard from all Standard readings.
13.2 Plot the Fluorescence Standard Curve with pmol of DNA on the xaxis and RFU on the y-axis.
13.3 Apply a linear fit to the Fluoresence Standard values and determine the Standard Curve equation.
13.4 Plot changes in RFU for each Sample on the y-axis vs. time (in min) on the x-axis and determine the slope (RFU/min) of the linear portion of the reaction curve.
13.5 Apply (RFU/min) to the fluorescence Standard Curve to obtain the activity (A) of the Samples (pmol/min).
13.6 Subtract Sample Background readings (ABC) from all Samples readings (AS) to obtain the corrected activity (AC), (AC= AS-ABC).

$$Sample\ Exonuclease\ activity\ = \frac{Ac}{V}\ \ X\ D\ (pmol/min/mL\ )$$

$$Sample\ specific\ activity = \frac{Ac}{(V\ x\ P)} XD\ \ (pmol/min/\mu g)$$

V = Sample volume added to the reaction well (µL)
D = Dilution factor
P = Protein concentration (µg/µL)
Ac = pmol/min (from linear range of the activity curve)

Unit definition:

One unit of Exonuclease activity is the amount of enzyme that can digest 1 µmol of DNA molecule per min at 37°C.

1 pmol/min/mL = 1 µU/µL
1 pmol/min/µg = 1 µU/µg

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

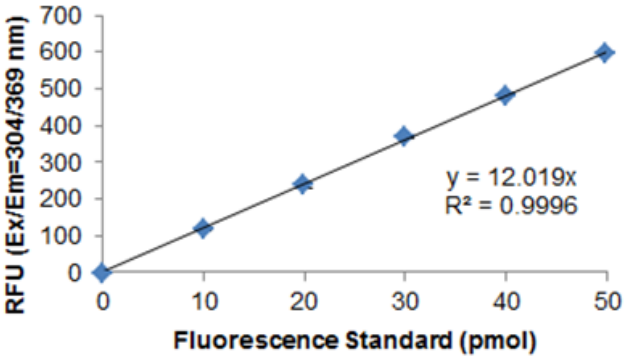


Figure 1. Fluorescence Standard Curve.

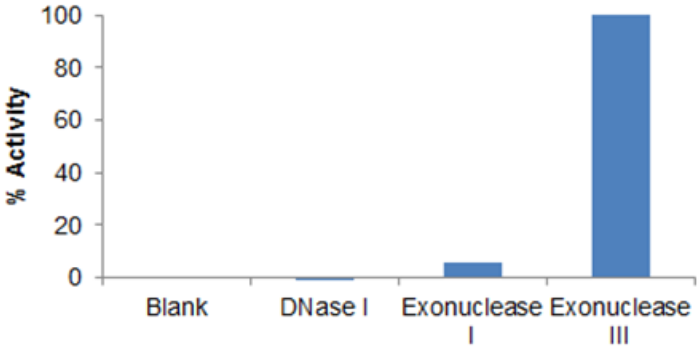


Figure 2. Specific activity of the probe to 3' to 5' Exonuclease (Exonucleae III) in contrast to DNAase I. Same units of proteins were added as defined by the commercially available values.

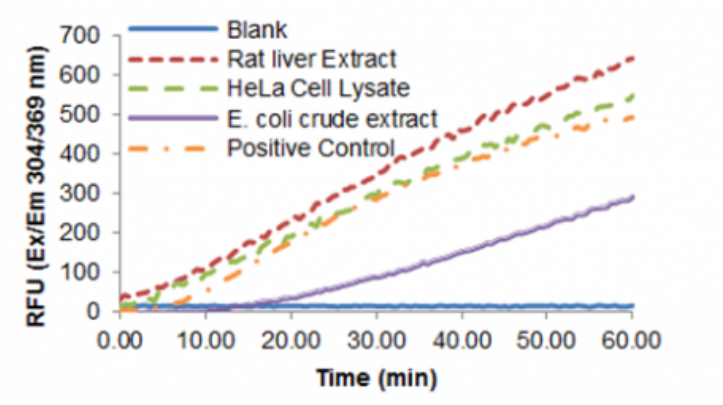


Figure 3. Kinetic curve rat liver extract (40.6 µg), HeLa cell lysate(12.2 µg) and *E.coli* (0.642 µg).

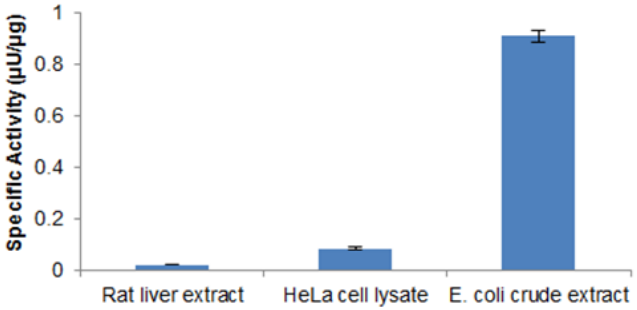


Figure 4. Specific activity from rat liver extract (0.0206 µU/µg). HeLa cell lysate (0.0841 µU/µg) and *E.coli* crude extract (0.9071 µU/µg). Assays performed according to the kit protocol.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)