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3' to 5' Exonuclease Activity Assay (Fluorometric)

rev 05/21

(Catalog # K175-100; 100 assays; Store at -20 °C)

I. Introduction:

Exonucleases remove a single nucleotide or an oligonucleotide at the 5' or 3' end. Exonuclease, such as Three Prime Repair Exonuclease 1 (TREX1), plays an important role in proofreading of DNA amplification and removal of damaged DNA. Deficiency of TREX1 enzyme has been shown to trigger autoimmune disease, Aicardia-Goutieres syndrome. TREX1 expression has been reported to correlate with cervical cancer cell growth and disease progression. In addition, 3' exonucleases are often used as tools in molecular biology such as site-directed mutagenesis and the generation of single stranded DNA probe. **BioVision's 3' to 5' Exonuclease Activity Assay Kit** 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.

Exonuclease Probe —**3' to 5' exonuclease**→ Fluorescent Product (Ex/Em = 304/369 nm)

II. Applications:

- Measurement of 3' to 5' exonuclease activity in biological samples
- Quality control of purified 3' to 5' exonuclease

III. Sample Type:

- Mammalian and bacterial cell culture
- Animal tissue

IV. Kit Contents:

Components	K175-100	Cap Code	Part Number
Exonuclease Assay Buffer	25 ml	WM	K175-100-1
Exonuclease Probe	1 vial	Red	K175-100-2
Half-area Plate	1 plate	--	K175-100-3
Fluorescence Standard (2 mM in DMSO)	100 µl	Yellow	K175-100-4
Exonuclease Positive Control	1 vial	Blue	K175-100-5

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge small vials prior to opening. Keep all components on ice while in use. Read the entire protocol before performing the assay.

- **Exonuclease Assay Buffer:** Warm to RT. Ready to use. Store at RT.
- **Exonuclease Probe:** Reconstitute with 110 µl Molecular Biology Grade Water. Aliquot and store at -20 ºC, protected from light. Avoid multiple freeze-thaw cycles.
- **Half-area Plate:** Ready to use. Store at RT.
- **Fluorescence Standard (2 mM in DMSO):** Warm to RT. Ready to use. Store at -20 °C.
- **Exonuclease Positive Control:** Reconstitute with 100 µl Exonuclease Assay Buffer. Aliquot and store at -20 ºC. Avoid multiple freeze-thaw cycles.

VII. 3' to 5' Exonuclease Activity Assay Protocol:

1. **Sample Preparation: For bacterial cell culture:** Collect bacterial cells from 1 ml culture by centrifugation at 4000 x g for 10 min. Discard the supernatant and resuspend with 100 µl Exonuclease Assay Buffer. Lyse the bacterial cells with a sonicator. Centrifuge the lysate at >10,000 x *g* and 4 °C for 10 min and collect the supernatant. **For mammalian cell culture:** Collect 500,000-1,000,000 mammalian cells by centrifugation. Resuspend the cell pellet in 100 µl Exonuclease Assay Buffer and rapidly homogenize the solution with a dounce homogenizer. Centrifuge the lysate at >10,000 x *g* and 4 °C for 10 min and collect the supernatant. **For animal tissue:** Rapidly homogenize 10 mg of tissue in 100 µl Exonulcease Assay Buffer with a Dounce homogenizer. Centrifuge the lysate at >10,000 x *g* and 4 °C for 10 min and collect the supernatant. Add 2 volumes of saturated ammonium sulfate solution into the supernatant (200 µl $(NH_4)_2SO_4$ for 100 µl of Sample). Place on ice for 30 min to precipitate the protein. Centrifuge at >10,000 x *g* and 4 °C for 10 min and collect the precipitate. Resuspend the precipitated protein in 100 µl Exonuclease Assay Buffer. Pipette up and down to make sure that the precipitated protein completely dissolves. **For Positive Control:** Add 5-10 µl of the Exonuclease Positive Control into a well in the Half-area Plate. **For all Samples:** Add 2-25 µl of the Sample in two parallel wells in the 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.

   Note: Exonuclease Buffer contains DTT. Reducing Agents compatible BCA protein assay kit (BioVision Cat # K818) should be used for Sample protein concentration measurement if you wish to express activity as µU/µg protein.

2. **Exonuclease Standard Curve:** Make 100 µM Standard solution by adding 50 µl of the 2 mM Fluorescence Standard into 950 µl of dH_2O. Make a 5 µM Standard solution by adding 10 µl of 100 µM Standard solution into 190 µl of dH_2O. Add 0, 2, 4, 6, 8, 10 µl of the 5 µM Standard solution into a series of wells in the half-area plate resulting in 0, 10, 20, 30, 40, 50 pmol of Standard/well. Adjust the volume to 50 µl/well with Exonuclease Assay Buffer.
3. **Reaction Mix:** 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:

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

Mix and add 25 µl of the Sample Reaction Mix to each well containing the Samples (S) and Positive Control. Add 25 µl of the Background Reaction Mix into the wells containing Sample Background Control (BC). Mix wells for 30-60 sec.

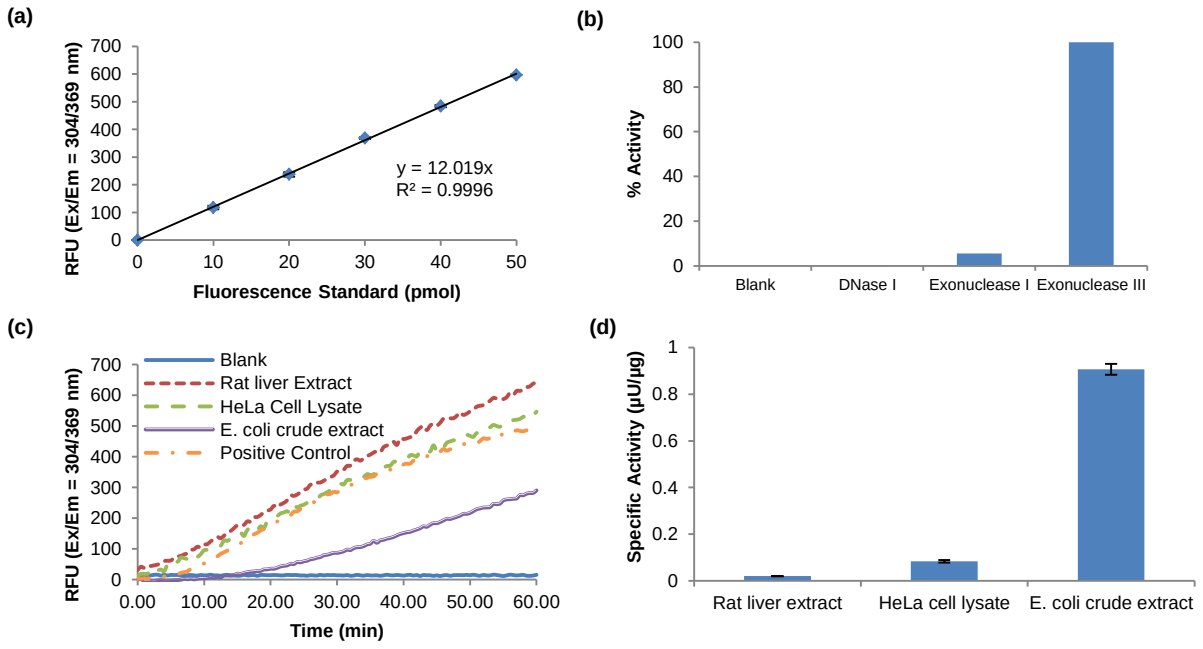
4. **Measurement:** Measure the fluorescence (Ex/Em = 304/369 nm) in kinetic mode every 30 sec for 30-60 min at 37 °C. Adjust GAIN/PMT setting of your fluorometer as necessary so that the Standard Curve readings are linear within the detection range of the instrument.
5. **Calculations:** Subtract 0 pmol Standard from all Standard readings. Plot the Fluorescence Standard Curve with pmol of DNA on the x-axis and RFU on the y-axis. Apply a linear fit to the Fluoresence Standard values and determine the Standard Curve equation. 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. Apply (RFU/min) to the fluorescence Standard Curve to obtain the activity (A) of the Samples (pmol/min). Subtract Sample Background readings (A_{BC}) from all Samples readings (A_S) to obtain the corrected activity (A_C), ($A_C = A_S - A_{BC}$).

Sample Exonuclease Activity = A_C/V x D (pmol/min/ml ≡ µU/µl)

Sample Specific Activity = A_C/(VxP) x D (pmol/min/µg ≡ µU/µg)

Where: **V** = Sample volume added into the reaction well (µl).
D = Dilution Factor
P = Protein concentration (µg/µl)
A_C= pmol/min (from the 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.



Figures: (a). Fluorescence Standard Curve. **(b).** Specificity of the probe to 3' to 5' exonuclease (Exonuclease III) in contrast to DNase I and Exonuclease I. Same units of proteins were added as defined by the commercially available values **(c).** Kinetic curve of Rat Liver Extract (40.6 µg), HeLa Cell Lysate (12.2 µg) and *E. coli* crude extract (0.642 µg). **(d).** Specific activity from Rat Liver Extract (0.0206 µU/µg), HeLa Cell Lysate (0.0841 µU/µg) and *E. coli* crude extract (0.907 µU/µg). Assays were performed according to the kit protocol.

VIII. Related Products:

DNA Quantification Assay (K539)
EZQuant™ dsDNA Quantitation Kit (Fluorometric) (K900)
EZQuant™ RNA Quantification Kit I (K1480)
EZQuant™ RNA Quantification Kit II (K1481)
EasyRNA™ Bacterial RNA Kit (K1351)
Yeast RNA Mini Kit (K1418)

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