User Guide

Benzonase III ELISA Kit

96-Well Plate

EZBNZ3-160K

EZBNZ3-160K5PK

Intended Use

This Benzonase III ELISA kit is used for the non-radioactive quantification of Benzonase. One kit is sufficient to measure 40 unknown samples in duplicate. This kit is for research use only. Not for use in diagnostic procedures.

Principles of Assay

This assay is a Sandwich ELISA based, sequentially, on:

- Capture of Benzonase molecules from samples to the wells of a microtiter plate coated with an anti-Benzonase antibody
- Washing of unbound materials from samples
- Binding of a second biotinylated anti-Benzonase antibody to the captured molecules
- Washing of unbound materials from samples
- Binding of streptavidin-horseradish peroxidase conjugate to the immobilized biotinylated antibodies
- Washing of excess free enzyme conjugates
- Quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3′,5,5′-tetramethylbenzidine.

The enzyme activity is measured spectrophotometrically by the increased absorbance at 450–590 nm after acidification of formed products. Since the increase in absorbance is directly proportional to the amount of captured Benzonase in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of Benzonase.

Reagents Supplied

Each kit is sufficient to run one 96-well plate and contains the following reagents:

Note: Store all reagents at 2-8 °C.

Reagents Supplied	Catalogue Number	Volume	Quantity
Microtiter Plate with 2 plate sealers	EP-BNZ3	---------	1 plate 2 sealers
Benzonase III Standard	E8160-K	100 µL	1 vial
Assay Buffer	EAB-BNZ	10 mL	1 bottle
10X Wash Buffer	EWB-HRP	50 mL	2 bottles
Benzonase III Detection Antibody	E1160-K	12 mL	1 bottle
Enzyme Solution (100X)	EHRP-BNZ	150 µL	1 bottle
Enzyme Solution Diluent	ED-BNZ	12 mL	1 bottle
Substrate Solution	ESS-TMBS1	12 mL	1 bottle
Stop Solution	ET-TMB	12 mL	1 bottle

Storage and Stability

Recommended storage for kit components is 2-8 °C.

All components are shipped and stored at 2-8 °C. Reconstituted standards and controls can be frozen for future use but repeated freeze/thaw cycles should be avoided. Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers.

Reagent Precautions

Sodium azide or Proclin™ has been added to some reagents as a preservative. Although the concentrations are low, Sodium azide and Proclin™ may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

Full Hazard Label

Ingredient	Component Number	Full Label	
Benzonase III Detection Antibody	E1160-K		Warning. Causes serious eye irritation. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. Do not breathe mist or vapours. Wash skin thoroughly after handling. Wear eye protection/ face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/attention if you feel unwell. If eye irritation persists: Get medical advice/attention. Dispose of contents/container to an approved waste disposal plant.
Assay Buffer	EAB-BNZ		Warning. Causes serious eye irritation. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. Do not breathe mist or vapours. Wash skin thoroughly after handling. Wear eye protection/ face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/attention if you feel unwell. If eye irritation persists: Get medical advice/attention. Dispose of contents/container to an approved waste disposal plant.
Enzyme Solution (100X)	EHRP-BNZ		Warning. May cause an allergic skin reaction. Avoid breathing mist or vapours. Wear protective gloves. IF ON SKIN: Wash with plenty of water. If skin irritation or rash occurs: Get medical advice/attention. Take off contaminated clothing and wash it before reuse. Dispose of contents/container to an approved waste disposal plant.

Ingredient	Component Number	Full Label	
Enzyme Solution Diluent	ED-BNZ		Warning. May cause an allergic skin reaction. Avoid breathing mist or vapours. Wear protective gloves. IF ON SKIN: Wash with plenty of water. If skin irritation or rash occurs: Get medical advice/attention. Take off contaminated clothing and wash it before reuse. Dispose of contents/container to an approved waste disposal plant.
10X HRP Wash Buffer	EWB-HRP		Warning. May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap.
Stop Solution	ET-TMB		Danger. May be corrosive to metals

Materials Required

(Not Provided)

- Multi-channel Pipettes and pipette tips: 5-50 µL and 50-300 µL
- Pipettes and pipette tips: 10-20 µL or 20-100 µL
- Reagent Reservoirs
- Polypropylene Microfuge Tubes
- Vortex Mixer
- De-ionized water
- Microtiter Plate Reader capable of reading absorbency at 450 nm
- Orbital Microtiter Plate Shaker
- Absorbent Paper or Cloth

Reagent Preparation

Benzonase III Standard Preparation

1. Take 5 µL of stock standard (5 ug/mL) added to 495 µL of Assay Buffer to make a 50 ng/mL standard concentration.
2. Next, take 10 µL of the 50 ng/mL standard concentration prepared above and add to 490 µL of Assay Buffer. This 1000 pg/mL standard concentration will be Standard 7 in the table below.
3. Label 6 polypropylene microfuge tubes as Std 6, Std 5, Std 4, Std 3, Std 2, Std 1.

4. Add 250 µL of Assay Buffer to each of the 6 tubes.
5. Prepare serial dilutions by adding 250 µL of the 1000 pg/mL standard concentration to the number 6 tube, mix well.
6. Transfer 250 µL of the number 6 standard to the number 5 tube, mix well.
7. Transfer 250 µL of the number 5 standard to the number 4 tube, mix well.
8. Transfer 250 µL of the number 4 standard to the number 3 tube, mix well.
9. Transfer 250 µL of the number 3 standard to the number 2 tube, mix well.
10. Transfer 250 µL of the number 2 standard to the number 1 tube, mix well.
11. The 0 standard (Background) will be Assay Buffer.

Note: Change tip for every dilution. Wet tip with standard before dispensing. Unused portions of reconstituted standard should be stored in small aliquots at ≤ -20 °C. Avoid multiple freeze/thaw cycles.

Tube #	Volume of Assay Buffer to Add	Volume of 50 ng/mL Standard to Add	Standard Concentration
Standard 7	490 µL	10 µL of 50 ng/mL standard concentration	1000 pg/mL

Tube #	Volume of Assay Buffer to Add	Volume of Standard to Add	Standard Concentration
Standard 6	250 µL	250 µL of Standard 7	500 pg/mL
Standard 5	250 µL	250 µL of Tube # 6	250 pg/mL
Standard 4	250 µL	250 µL of Tube # 5	125 pg/mL
Standard 3	250 µL	250 µL of Tube # 4	62.5 pg/mL
Standard 2	250 µL	250 µL of Tube # 3	31.25 pg/mL
Standard 1	250 µL	250 µL of Tube # 2	15.63 pg/mL

Preparation of Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 100 mL of 10X Wash Buffer (two bottles) with 900 mL deionized water. Store unused portion at 2-8 °C for up to one month.

Preparation of Enzyme Solution

Add 120 µL of 100X enzyme solution to the bottle containing 12 mL of enzyme solution diluent. Mix well. Store unused portion at 2-8 °C for up to one month.

Benzonase III ELISA Assay Procedure

Warm all reagents to room temperature before setting up the assay.

1. Remove the required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch and stored at 2-8 °C. Assemble the strips in an empty plate holder. Add 300 µL diluted Wash Buffer to each well of the plate. Decant Wash Buffer and remove the residual volume by inverting the plate and tapping it smartly onto absorbent towels several times. Repeat wash procedure 2 additional times. **Do not let wells dry before proceeding to the next step.** If an automated machine is used for the assay, follow the manufacturer's instructions for all washing steps described in this protocol.
2. Add 50 µL Assay Buffer to each of the Blank.
3. Add 50 µL Standards to the appropriate wells.
4. Add 50 µL of sample to the appropriate wells.
5. Cover the plate with plate sealer and incubate at room temperature for 2 hours on an orbital microtiter plate shaker set to rotate at moderate speed, about 400-500 rpm.
6. Remove plate sealer and decant reagents from the plate. Tap as before to remove residual volume in well. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.
7. Add 100 µL Detection Antibody to each well. Re-cover plate with sealer and incubate at room temperature for 1 hour on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400-500 rpm.
8. Remove plate sealer and decant reagents from the plate. Tap as before to remove residual volume in well. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.
9. Add 100 µL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 minutes on the microtiter plate shaker.
10. Remove sealer, decant reagents from the plate and tap plate to remove the residual volume. Wash wells 5 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

11. Add 100 µL of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for **approximately** 30 minutes. Blue color should be formed in wells of the Benzonase III standards with intensity proportional to increasing concentrations of Benzonase.

    Note: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.

12. Remove sealer and add 100 µL Stop Solution (CAUTION: CORROSIVE SOLUTION) and gently shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn to yellow after acidification. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. Read absorbance at 450 nm and 590 nm in a plate reader within 5 minutes and ensure that there are no air bubbles in any well. Record the difference of absorbance units. The absorbance of the highest Benzonase III standard should be approximately 2.0-3.0, or not to exceed the capability of the plate reader used.

    Note: When sample volumes assayed differ from 50 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (For example, if 25 µL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 50 µL, compensate for the volume deficit with Assay Buffer.

Assay Procedure for Benzonase III ELISA Kit

Well #	Step 1		Step 2	Step 3-4	Step 5-6	Step 7	Step 7-8	Step 9	Step 9-10	Step 11-12	
			Assay Buffer	Standards/ Samples		Detection Antibody		Enzyme Solution		Substrate	Stop
A1, B1	Wash plate 3X with 300 µL 1X Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels.		50 µL	--	Seal, Agitate, Incubate 2 hours at Room Temperature on a plate shaker. Wash 3X with 300 µL Wash Buffer.	100 µL	Seal, Agitate, Incubate 1 hours at Room Temperature on a plate shaker. Wash 3X with 300 µL Wash Buffer.	100 µL	Seal, Agitate, Incubate 30 minutes at Room Temperature on a plate shaker. Wash 5X with 300 µL Wash Buffer.	100 µL	100 µL
C1, D1			--	50 µL of Standard 1							
E1, F1			--	50 µL of Standard 2						Seal, Agitate, Incubate for 30 minutes at Room Temperature.	
G1, H1			--	50 µL of Standard 3							
A2, B2			--	50 µL of Standard 4							
C2, D2			--	50 µL of Standard 5							
E2, F2			--	50 µL of Standard 6							
G2, H2			--	50 µL of Standard 7							
A3, B3			--	50 µL of Sample							
C3, D3		--		50 µL of Sample							
E3, F3		--		--							
G3, H3, etc.		--		--							Read Absorbance at 450 nm and 590 nm.

Microtiter Plate Arrangement

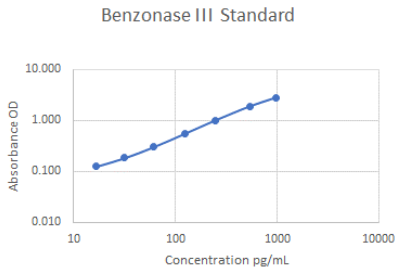
Benzonase III ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Sample 1									
B	Standard 1	Standard 1	Sample 1									
C	Standard 2	Standard 2	Sample 2									
D	Standard 3	Standard 3	Sample 2									
E	Standard 4	Standard 4										
F	Standard 5	Standard 5										
G	Standard 6	Standard 6										
H	Standard 7	Standard 7										

Assay Characteristics

Sensitivity

The lower limit of quantitation (LLOQ) of Benzonase III assay is 15.63 pg/mL using Belysa Immunoassay Analysis software from MilliporeSigma. LLOQ is calculated by back interpolation of the standard point that provides CV≤20% and recovery ±20% of the expected.



Precision

Mean Intra-assay precision is calculated from the results of twenty replicates each of the two different concentrations of Benzonase in a single assay. The mean inter-assay precision is generated from the results of eight separate assays with duplicate samples in each assay for the two different concentrations of Benzonase.

Intra-Assay Variation

	Mean Benzonase Levels (pg/mL)	Intra-Assay %CV
1	478.9	7.10
2	88.24	7.95

Inter-Assay Variation

	Mean Benzonase Levels (pg/mL)	Inter-Assay %CV
1	392.5	7.24
2	67.56	6.51

Spike Recovery of Benzonase in Assay Samples

Varying amounts of Benzonase were added to assay buffer and the resulting Benzonase content of each sample was assayed by Benzonase III ELISA.

The recovery = [(observed- Basal / (spiked Benzonase concentration)] x 100%

Sample	Spiked Concentration of Benzonase Added (pg/mL)	Concentration Observed in the assay (pg/mL)	Recovery %
1	62.5	209.4	125
	125	292.2	129
	250	434.9	121
2	62.5	149.2	129
	125	231.8	130
	250	379.2	124
3	62.5	114.7	130
	125	191.2	126
	250	333.5	120
4	62.5	211.2	121
	125	289.5	123
	250	428.3	117
5	62.5	144.3	125
	125	222.5	125
	250	358.3	117
Average			124

Linearity of Dilution

5 samples with the indicated sample volumes were assayed. Neat sample volumes of 50 µL, 25 µL, 12.5 µL, and 6.25 µL in a 50 µL total sample volume represents dilution factors of 1, 2, 4, and 8, respectively. Required amounts of Assay Buffer were added to compensate for the lost volumes below 50 µL.

Dilution linearity = (observed/expected) x 100%

Observed = mean calculated dilution corrected concentration at each dilution

Expected = mean calculated concentration of the neat sample

Sample	Neat Sample volume in 50µL total volume	Mean (pg/mL)	Dilution Corrected (pg/mL)	Linearity %
1	50	551.2	551.2	
	25	258.2	516.3	94
	12.5	115.7	462.7	84
	6.25	62.7	501.8	91
2	50	235.1	235.1	
	25	109.3	218.5	93
	12.5	54.1	216.2	92
	6.25	28.7	229.3	98
3	50	531.7	531.7	
	25	250.8	501.5	94
	12.5	115.6	462.2	87
	6.25	63.1	504.7	95
4	50	246.8	246.8	
	25	111.9	223.7	91
	12.5	54.3	217.2	88
	6.25	29.2	233.3	95
5	50	101.3	101.3	
	25	48.6	97.2	96
	12.5	24.5	97.8	97
	6.25	14.0	111.9	111
Average				94

Troubleshooting

- To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
- Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
- Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
- Avoid cross contamination of any reagents or samples to be used in the assay.
- Make sure all reagents and samples are added to the bottom of each well.
- Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
- Remove any air bubbles formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
- High signal in background or blank wells could be due to:
- cross well contamination by standard solution or sample, or
- inadequate washing of wells with Wash Buffer, or
- overexposure to light after substrate has been added

Product Ordering

Products are available for online ordering at SigmaAldrich.com.

Replacement Reagents

Reagents	Catalogue Number
Microtiter Plate with 2 plate sealers	EP-BNZ3
Benzonase III Standard	E8160-K
Assay Buffer	EAB-BNZ
10X Wash Buffer	EWB-HRP
Benzonase III Detection Antibody	E1160-K
Enzyme Solution (100X)	EHRP-BNZ
Enzyme Solution Diluent	ED-BNZ
Substrate Solution	ESS-TMBS1
Stop Solution	ET-TMB
Benzonase III ELISA (5 pack bulk)	EZBNZ3-160K5PK

Notice

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