User Guide

Benzonase® ST ELISA with ZooMAb® Antibodies

96-Well Plate

EZBNZST-185K

EZBNZST-185K5PK

Intended Use

This Benzonase® ST ELISA with ZooMAb® Antibodies kit is used for the quantification of Benzonase® ST. One kit is sufficient to measure 38 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® ST molecules from samples to the wells of a microtiter plate coated with ZooMAb® rabbit anti-Benzonase® ST antibody.
- Washing of unbound materials from samples.
- Binding of a second biotinylated ZooMAb® rabbit anti-Benzonase® ST 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 nm–590 nm after acidification of formed products. Since the increase in absorbance is directly proportional to the amount of captured Benzonase® ST 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® ST.

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	EP185	-	1 plate 2 sealers
Benzonase® ST Standard	E8185-K	Lyophilized	1 vial
Benzonase® ST Quality Controls 1 and 2	E6185-K	Lyophilized	1 vial each
Assay Buffer	EAB180	10 mL	1 bottle
10X Wash Buffer	EWB-HRP180	50 mL	2 bottles
Benzonase® ST Detection Antibody	E1185-K	12 mL	1 bottle
Enzyme Solution (100x)	EHRP-185	150 uL	1 bottle
Enzyme Solution Diluent	ED-180	12 mL	1 bottle
Substrate Solution	ESS-TMB180	12 mL	1 bottle
Stop Solution	ET-TMB180	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.

Ingredient	Catalogue No.	Label	
Benzonase® ST Standard	E8185-K		**Danger.** Harmful if swallowed, in contact with skin or if inhaled. Causes serious eye damage. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. May cause damage to organs Brain through prolonged or repeated exposure if swallowed. Harmful to aquatic life with long lasting effects. Do not breathe dust. Wash skin thoroughly after handling. Do not eat, drink, or smoke when using this product. Use only outdoors or in a well-ventilated area. Avoid release to the environment. Wear protective gloves/ protective clothing/ eye protection/ face protection. IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth. IF ON SKIN: Wash with plenty of water. Call a POISON CENTER/ doctor if you feel unwell. IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor if you feel unwell. 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. Wash contaminated clothing before reuse. Dispose of contents/ container to an approved waste disposal plant.

Ingredient	Catalogue No.	Label	
Benzonase® ST Quality Control 1 & 2	E6185-K		**Danger.** Harmful if swallowed, in contact with skin or if inhaled. Causes serious eye damage. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. May cause damage to organs Brain through prolonged or repeated exposure if swallowed. Harmful to aquatic life with long lasting effects. Do not breathe dust. Wash skin thoroughly after handling. Do not eat, drink, or smoke when using this product. Use only outdoors or in a well-ventilated area. Avoid release to the environment. Wear protective gloves/ protective clothing/ eye protection/ face protection. IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth. IF ON SKIN: Wash with plenty of water. Call a POISON CENTER/ doctor if you feel unwell. IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor if you feel unwell. 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. Wash contaminated clothing before reuse. Dispose of contents/ container to an approved waste disposal plant.
Enzyme Solution (100x)	EHRP-185		**Danger.** May cause an allergic skin reaction. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing mist or vapours. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves. In case of inadequate ventilation wear respiratory protection. IF ON SKIN: Wash with plenty of soap and water. IF INHALED: If breathing is difficult, remove person to fresh air and keep comfortable for breathing. If skin irritation or rash occurs: Get medical advice/ attention. If experiencing respiratory symptoms: Call a POISON CENTER/ doctor. Wash contaminated clothing before reuse. Dispose of contents/ container to an approved waste disposal plant.

Ingredient	Catalogue No.	Label	
Assay Buffer	EAB180		**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.
Benzonase®S T Detection Antibody	E1185-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.
Stop Solution	ET-TMB180		**Warning.** May be corrosive to metals. Keep only in original container. Absorb spillage to prevent material damage. Store in corrosive resistant container with a resistant inner liner.

Additional Supplies Required (Not Provided)

Equipment

- Microtiter Plate Reader capable of reading absorbency at 450-590 nm
- Orbital Microtiter Plate Shaker
- Belysa® Immunoassay Curve Fitting Software (40-122)

Supplies

- Multi-channel Pipettes and pipette tips: 5 µL-50 µL and 50 µL-300 µL
- Pipettes and pipette tips:20 µL-100 µL
- Reagent Reservoirs
- Polypropylene Microfuge Tubes
- Vortex Mixer
- Absorbent Paper

Reagents

- De-ionized water

Reagent Preparation

Benzonase® ST Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the Benzonase® ST Standard with 400 µL distilled or de-ionized water. Invert and mix gently, let sit for 5-10 minutes then mix well.
2. Label 6 polypropylene microfuge tubes as Std 6, Std 5, Std 4, Std 3, Std 2, Std 1.
3. Add 200 µL of Assay Buffer to each of the 6 tubes.
4. Prepare serial dilutions by adding 200 µL of the reconstituted standard to the Std 6 tube, mix well.
5. Transfer 200 µL of Std 6 to the Std 5 tube, mix well.
6. Transfer 200 µL of Std 5 to the Std 4 tube, mix well.
7. Transfer 200 µL of Std 4 to the Std 3 tube, mix well.
8. Transfer 200 µL of Std 3 to the Std 2 tube, mix well.
9. Transfer 200 µL of Std 2 to the Std 1 tube, mix well.
10. The 0 pg/mL 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 Deionized Water to Add	Volume of Standard to Add	Standard Stock Concentration
Reconstituted standard (Tube 7)	400 uL	0	1000 pg/mL

Tube #	Volume of Assay Buffer to Add	Volume of Standard to Add	Standard Concentration (pg/mL)
Tube 6	200 µL	200 µL of reconstituted standard	500pg/mL
Tube 5	200 µL	200 µL of Tube 6	250pg/mL
Tube 4	200 µL	200 µL of Tube 5	125 pg/mL
Tube 3	200 µL	200 µL of Tube 4	62.5 pg/mL
Tube 2	200 µL	200 µL of Tube 3	31.25 pg/mL
Tube 1	200 µL	200 µL of Tube 2	15.6 pg/mL

Benzonase® ST Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Reconstitute each Benzonase® ST Quality Control 1 and Quality Control 2 with 250 µL distilled or de-ionized water and gently invert to ensure complete hydration. Unused portions of the reconstituted Quality Controls should be stored in small aliquots at ≤−20 °C. Avoid further freeze/thaw cycles.

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 120µL 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.

Preparation of Samples

Some dilution of samples will be necessary depending on the composition of the formulation buffer. Samples can be diluted in assay buffer. Extra assay Buffer (EAB180) is available for purchase.

Benzonase® ST ELISA with ZooMAb® Antibodies 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 in duplicate 50 µL Assay Buffer to each of the Blank, Sample, Control, and Standard wells.
3. Add 50uL of Assay Buffer to each of the Blank sample wells.
4. Add in duplicate 50 µL Standards or Controls to the appropriate wells.
5. Add in duplicate 50 µL of sample to the appropriate wells.
6. 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 to 500 rpm.
7. 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.
8. 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.
9. 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.
10. 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.
11. Remove sealer, decant reagents from the plate and tap plate to remove the residual volume. Wash wells 6 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

12. Add 100 µL of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for approximately 13-18 minutes. Blue color should be formed in wells of the Benzonase® ST standards with intensity proportional to increasing concentrations of Benzonase® ST.

13. 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.

14. 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® ST 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 50µL, compensate for the volume deficit with Assay Buffer.

Benzonase® ST ELISA with ZooMAb® Antibodies Assay Procedure

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/ QCs/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.	100 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 6X with 300 µL Wash Buffer.	100 µL	Seal, Agitate, Incubate for 13-18 minutes at Room Temperature.	100 µL	Read Absorbance at 450 nm and 590 nm.
C1, D1		50 µL	50 µL of Tube 1								
E1, F1		50 µL	50 µL of Tube 2								
G1, H1		50 µL	50 µL of Tube 3								
A2, B2		50 µL	50 µL of Tube 4								
C2, D2		50 µL	50 µL of Tube 5								
E2, F2		50 µL	50 µL of Tube 6								
G2, H2		50 µL	50 µL of Tube 7								
A3, B3		50 µL	50 µL of QC 1								
C3, D3		50 µL	50 µL of QC 2								
E3, F3		50 µL	50 µL of Sample								
G3, H3, etc.		50 µL	50 µL of sample								

Microtiter Plate Arrangement

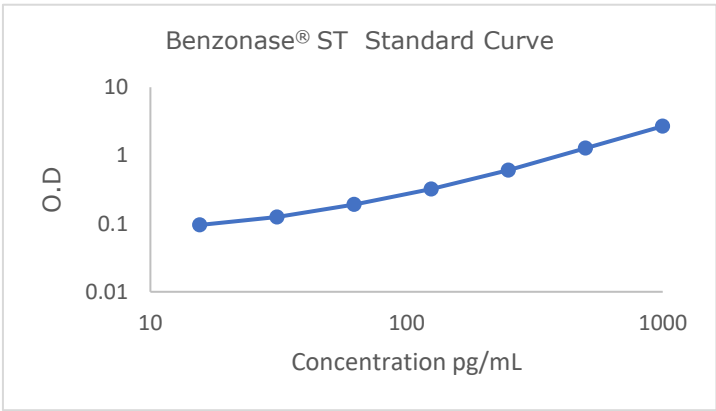
Benzonase® ST ELISA with ZooMAb® Antibodies

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Tube 4	QC 1	Etc								
B	Blank	Tube 4	QC 1									
C	Tube 1	Tube 5	QC 2									
D	Tube 1	Tube 5	QC 2									
E	Tube 2	Tube 6	Sample 1									
F	Tube 2	Tube 6	Sample 1									
G	Tube 3	Reconstituted Standard (Tube 7)	Sample 2									
H	Tube 3	Reconstituted Standard (Tube 7)	Sample 2									

Assay Characteristics

Sensitivity

The lower limit of quantitation (LLOQ) of the Benzonase® ST assay is 15.6 pg/mL using Belysa Immunoassay Analysis software from Millipore Sigma. LLOQ is calculated by back interpolation of the standard point that provides CV≤ 20% and recovery ± 20%.



Precision

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

Intra-Assay Variation

	Mean Benzonase® ST Levels pg/mL)	Intra-Assay %CV
1	237.7	3.9%
2	73.9	3.4%

Inter-Assay Variation

	Mean Benzonase® ST Levels (pg/mL)	Inter-Assay %CV
1	238.9	3.5%
2	68.8	5.3%

Spike Recovery of Benzonase® ST in Assay Samples

Varying amounts of Benzonase® ST were added to assay buffer and the resulting Benzonase® ST concentration of each sample was assayed by Benzonase® ST ELISA with ZooMAb® Antibodies.

The recovery=[(observed-Basal/(spiked of Benzonase® ST concentration)]x100%

Sample	Spiked concentration of Benzonase® ST Added (pg/mL)	Concentration Observed in the assay (pg/mL)	Recovery
1	0	22.1	
	62.5	72	80%
	125	124.9	82%
	250	241.5	88%
2	0	38.4	
	62.5	90.1	83%
	125	147.3	87%
	250	280	97%
3	0	75.6	
	62.5	131.9	90%
	125	187.7	90%
	250	315.7	96%
4	0	32.3	
	62.5	82.5	82%
	125	134.4	82%
	250	258.7	91%
5	0	16.8	
	62.5	67.2	81%
	125	122.1	84%
	250	249.0	93%
Average			87%

Linearity of Sample Dilution

Five (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.
Mean=mean calculated concentration of the neat sample

Dilution Corrected=Mean*dilution factor % Linearity=Dilution corrected value at each dilution factor/dilution corrected value of non-diluted sample*100.

Sample	Neat sample volume in 50uL total volume (µL)	Mean (pg/mL)	Dilution Corrected (pg/mL)	Linearity %
1	50	817	817	
	25	390.9	781.8	96%
	12.5	192.6	770.4	94%
	6.25	106.0	847.9	104%
2	50	418.3	418.3	
	25	204	408	98%
	12.5	83.7	334.8	80%
	6.25	56.1	449.1	107%
3	50	830.4	830.4	
	25	406	812.0	98%
	12.5	187.3	749.1	90%
	6.25	111.7	893.4	108%
4	50	202.4	202.4	
	25	100.9	201.8	100%
	12.5	50.1	200.4	99%
	6.25	25.0	199.6	99%
5	50	108.7	108.7	
	25	50.4	100.9	93%
	12.5	25	99.9	92%
	6.25	13.6	108.4	100%
Average				97%

Quality Controls

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert, or available at our website SigmaAldrich.com.

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
Benzonase®ST ELISA Plate	EP185
10X HRP Wash Buffer Concentrate	EWB-HRP180
Benzonase®ST ELISA Standard	E8185-K
Benzonase®ST Quality Controls 1 & 2	E6185-K
Assay Buffer	EAB180
Benzonase®ST Detection Antibody	E1185-K
Enzyme Solution (100x)	EHRP-185
Enzyme Solution Diluent	ED-180
Substrate Solution	ESS-TMB180
Stop Solution	ET-TMB180
Benzonase® ST ELISA with ZooMAb® Antibodies (5 Pack Bulk)	EZBNZST-185K5PK

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