

## Bradykinin ELISA kit

Catalog # ADI-900-206

96 Well Enzyme-Linked Immunosorbent Assay Kit

For use with plasma, serum and urine



Reagents require separate storage conditions.



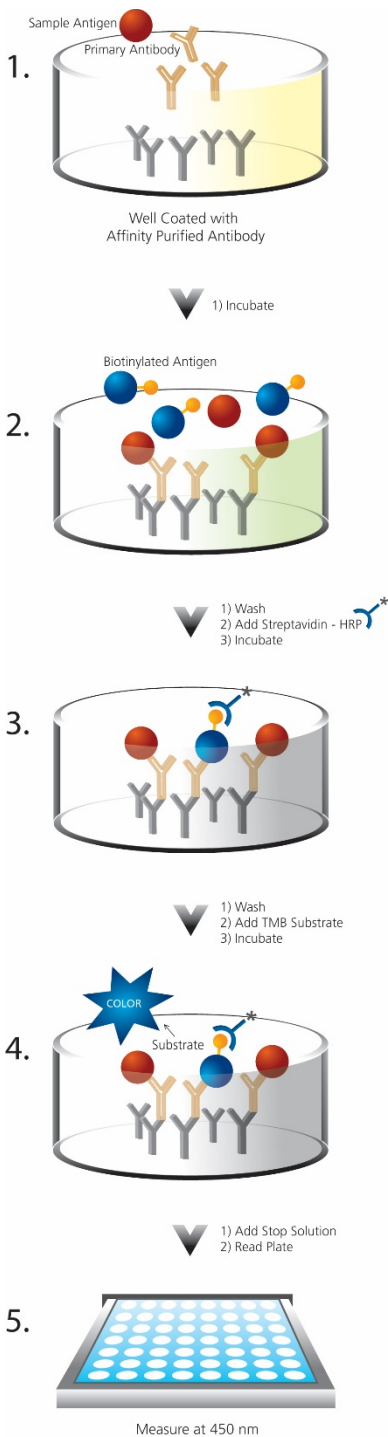
Check our website for additional protocols, technical notes and FAQs.



For proper performance, use the insert provided with each individual kit received.

### Table of Contents

|           |  |
|-----------|--|
| <u>2</u>  | <u>Introduction</u>                      |
| <u>2</u>  | <u>Principle</u>                         |
| <u>3</u>  | <u>Materials Supplied</u>                |
| <u>4</u>  | <u>Storage</u>                           |
| <u>4</u>  | <u>Materials Needed but Not Supplied</u> |
| <u>5</u>  | <u>Sample Handling</u>                   |
| <u>6</u>  | <u>Sample Recoveries</u>                 |
| <u>7</u>  | <u>Reagent Preparation</u>               |
| <u>8</u>  | <u>Assay Procedure</u>                   |
| <u>9</u>  | <u>Calculation of Results</u>            |
| <u>10</u> | <u>Typical Results</u>                   |
| <u>11</u> | <u>Performance Characteristics</u>       |
| <u>13</u> | <u>References</u>                        |
| <u>16</u> | <u>Limited Warranty</u>                  |



## Introduction

The Bradykinin Enzyme-Linked Immunosorbent Assay (ELISA) kit is a complete kit for the quantitative determination of Bradykinin in plasma, serum and urine. Please read the entire kit insert before performing this assay.

Bradykinin was discovered in 1949 as a substance generated from a globulin precursor in plasma by the action of proteases. Its name indicates that it causes a slow movement of the gut<sup>1</sup>. As early as 1909 it was noted that substances found in urine, which were later identified as kinins, have hypotensive actions<sup>2</sup>.

Kinins are effectors of vasodilation, vascular permeability, NO release and arachidonic acid mobilization. They are important regulators of blood pressure, kidney function and heart function, and they are also involved in inflammation<sup>2,3,5</sup>.

Bradykinin is generated from the blood globulin kininogen HK, by the action of the kallikrein system in blood (related to the blood clotting cascade) but can also be generated in other tissues and organs<sup>3</sup>. Besides kallikrein, other proteases such as plasmin may also release bradykinin. Several peptidases can degrade kinins, including Angiotensin Converting Enzyme (ACE), a metalloproteinase which converts Angiotensin I to Angiotensin II and destroys bradykinin<sup>3,5</sup>. Plasma Bradykinin is rapidly degraded to a smaller stable peptide (BK1-5) form<sup>4</sup>.

## Principle

- Standards and samples are added to wells coated with a GxR IgG antibody. A blue solution of Bradykinin conjugated to biotin is then added to the wells.
- A yellow solution of rabbit polyclonal antibody to Bradykinin is then added, and the plate is incubated at room temperature. During this incubation the antibody binds, in a competitive manner, the Bradykinin in the sample or conjugate. The plate is then washed, leaving only bound Bradykinin.
- A solution of streptavidin conjugated to horseradish peroxidase is added to each well, to bind the biotinylated Bradykinin. The plate is again incubated.
- The plate is washed to remove excess HRP conjugate. TMB substrate solution is added. An HRP-catalyzed reaction generates a blue color in the solution.
- Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450 nm. The amount of signal is inversely proportional to the level of Bradykinin in the sample.



Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.



The standard should be handled with care due to the known and unknown effects of the antigen.



Protect substrate from prolonged exposure to light.



Stop solution is caustic. Keep tightly capped.

## Materials Supplied

- 1. Assay Buffer 16**  
50 mL, Product No. 80-2874  
Tris buffer containing proteins and preservative
- 2. Bradykinin Standard**  
Product No. 80-2382  
Two vials containing 30,000pg lyophilized Bradykinin
- 3. Goat anti-Rabbit IgG Microtiter Plate**  
One plate of 96 wells, Product No. 80-0060  
A clear plate of break-apart strips coated with a goat anti-rabbit polyclonal antibody
- 4. Bradykinin Antibody**  
5 mL, Product No. 80-2380  
A yellow solution of polyclonal antibody to Bradykinin
- 5. Bradykinin Conjugate**  
5 mL, Product No. 80-2381  
A blue solution of biotinylated Bradykinin
- 6. Streptavidin-HRP**  
Product No. 80-1896  
One vial containing 12.5 µg of lyophilized streptavidin conjugated to horseradish peroxidase.
- 7. Wash Buffer Concentrate**  
27 mL, Product No. 80-1286  
Tris buffered saline containing detergents
- 8. TMB Substrate**  
Product No. 80-0350  
Two bottles containing 10 mL each  
A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide
- 9. Stop Solution 2**  
10 mL, Product No. 80-0377  
A 1N solution of hydrochloric acid in water
- 10. Bradykinin Assay Layout Sheet**  
1 each, Product No. 30-0302
- 11. Plate Sealer**  
2 each



Reagents require separate storage conditions.

## Storage

All components of this kit, **except the Standard**, should be stored at 4°C upon receipt. The Standard should be stored at **-20°C**. Shipping conditions may not reflect storage conditions.

## Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Precision pipets for volumes between 5  $\mu\text{L}$  and 1000  $\mu\text{L}$ .
3. Repeater pipet for dispensing 50  $\mu\text{L}$  and 200  $\mu\text{L}$ .
4. Disposable beakers for diluting buffer concentrates.
5. Graduated cylinders.
6. Microplate shaker.
7. Lint-free paper toweling for blotting.
8. Microplate reader capable of reading at 450 nm.
9. Graph paper for plotting the standard curve.



If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

## Sample Handling

The assay is suitable for the measurement of Bradykinin in plasma, serum and urine. Due to the conserved nature of Bradykinin between species, this kit is not species specific. However, samples containing rabbit IgG will interfere in the assay due to the goat anti-rabbit IgG coated plate. Prior to use, frozen samples should be brought to 4°C and centrifuged, if necessary, to isolate residual debris. A minimum 1:16 dilution is required for plasma, urine and serum samples. This is the minimum recommended dilution necessary to remove matrix interference in the assay. Due to differences in individual samples, users must determine the optimal sample dilution for their particular experiments.

**Note: The short half-life of Bradykinin may lead to variability in serum results.**

### Protocol for Plasma

1. Collect whole blood in an ice cold tube containing Sodium EDTA.
2. Mix blood in a ratio of 1:4 with ice cold ethanol.
3. Centrifuge at 1000 x g for 15 minutes at 4°C.
4. Remove ethanol prepared plasma to a clean plastic tube.
5. Sample should be divided into aliquots and frozen within 2 hours of collection at or below -20°C. Samples may be stored frozen for up to 2 weeks or proceed with the sample preparation.
6. Dry down sample and reconstitute with the provided assay buffer prior to use in this assay.

### Protocol for Urine

1. Collect spontaneous or 24 hour urine in a bottle containing 10 – 15mL of 6N HCl as a preservative.
2. Urine should be mixed in a ratio of 1:4 with glacial acetic acid.
3. Centrifuge at 1500 x g for 30 minutes at 4°C.
4. Remove lower phase and transfer to a clean plastic tube.
5. Using the material collected in step 4, repeat steps 3 and 4 twice, adjusting centrifuge time to 15 minutes.
6. Sample may be divided into aliquots and stored at or below -20°C, or proceed with the sample preparation.
7. Dry down sample and reconstitute with the included assay buffer prior to use in this assay.

### Protocol for Serum

1. Collect whole blood in appropriate serum tubes
2. Incubate upright at room temperature for 30-45 minutes to allow clotting to occur.
3. Centrifuge at 1000 x g for 15 minutes at 4°C. Do not use brake.
4. Without disturbing the cell layer, place supernatant into clean tube containing protease inhibitor cocktail to a final concentration of 0.05% and PMSF to a final concentration of 1mM.
5. The supernatant may be divided into aliquots and stored at or below -20°C, or used immediately in the assay.
6. Samples may be stored for up to two weeks.
7. Avoid repeated freeze-thaw cycles.

## Sample Recoveries

Individually diluted samples were prepared to read within the dynamic range of the assay. Next recombinant Bradykinin was spiked into these samples at three different concentrations. Endogenous Bradykinin was subtracted from the spiked values and the average recovery in each of the spiked matrices was compared to the recovery of identical spikes in the assay buffer. The mean and the range percent recovery at the three different concentrations are indicated below for each matrix.

| Sample Matrix         | Dilution | Spike Concentration | Recovery of Spike (Range) |
|-----------------------|----------|---------------------|---------------------------|
| Human Plasma<br>(n=5) | 1:16     | 20000 pg/mL         | 130% (113-155)            |
|                       |          | 2000 pg/mL          | 102% (101-104)            |
|                       |          | 100 pg/mL           | 98% (14-148)              |
| Human Urine<br>(n=3)  | 1:16     | 2000 pg/mL          | 99% (97-102)              |
|                       |          | 100 pg/mL           | 88% (81-100)              |
|                       |          | 10 pg/mL            | 139% (58-279)             |
| Human Serum<br>(n=4)  | 1:64     | 2000 pg/mL          | 103% (94-112)             |
|                       |          | 100 pg/mL           | 123% (48-184)             |
|                       |          |                     |                           |



Glass or polypropylene tubes may be used for standard preparation. Avoid polystyrene.

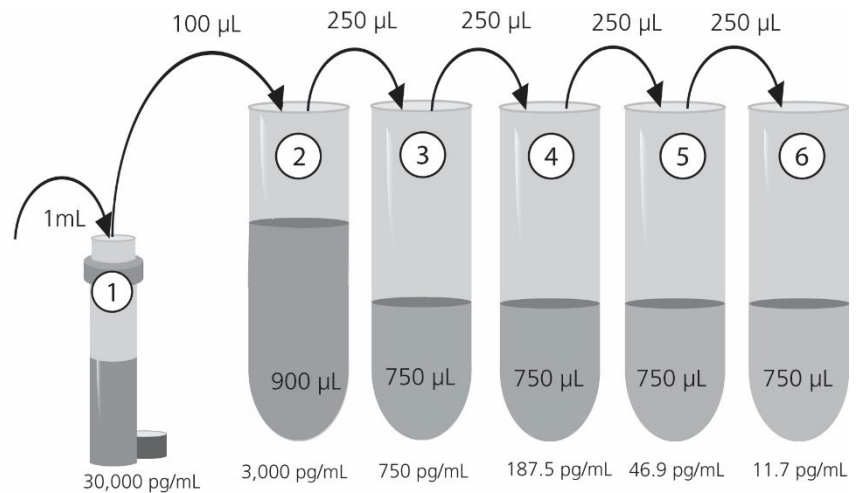
## Reagent Preparation

### 1. Wash Buffer

Prepare the wash buffer by diluting 5 mL of the supplied Wash Buffer Concentrate with 95 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

### 2. Bradykinin Standard

Reconstitute 1 vial of Bradykinin standard with 1 mL of the assay buffer. This is standard 1. Vortex to ensure the entire cake is dissolved. Label five 12 x 75 mm tubes #2 through #6. Pipet 900  $\mu$ L of the assay buffer into tube #2, pipet 750  $\mu$ L of the assay buffer into tube #3. Pipet 750  $\mu$ L of assay buffer into tubes #4 through #6. Remove 100  $\mu$ L from the reconstituted standard vial and add to tube #2, this is standard #2. Vortex thoroughly. Add 250  $\mu$ L from tube #2 to tube #3. Vortex thoroughly. Continue this for tubes #4 through #6.



**Diluted standards should be used within 30 minutes of preparation.** The concentrations of Bradykinin in the tubes are labeled above.

### 3. Streptavidin-HRP

Reconstitute 1 vial of Streptavidin-HRP with 250  $\mu$ L of deionized water and vortex thoroughly. Store at 4°C for up to 3 months. For prolonged storage, divide into aliquots and freeze at -20°C. Avoid repeated freeze/thaw cycles. Prepare the working concentration by diluting stock 1:1000 in the assay buffer.

**Do not store diluted Streptavidin-HRP.**



Bring all reagents to room temperature for at least 30 minutes prior to opening.



Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.



All standards and samples should be run in duplicate.



Add the reagents to the side of the well to avoid contamination.



Prior to the addition of substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in assay results.

## Assay Procedure

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove the wells not needed for the assay and return them, with the desiccant, to the mylar bag and seal. Store unused wells at 4°C.

1. Pipet 150  $\mu\text{L}$  of the assay buffer into the NSB (non-specific binding) wells.
2. Pipet 100  $\mu\text{L}$  of the assay buffer into the Bo (0 ng/mL standard) wells.
3. Pipet 100  $\mu\text{L}$  of Standards #1 through #6 to the bottom of the appropriate wells.
4. Pipet 100  $\mu\text{L}$  of the samples to the bottom of the appropriate wells.
5. Pipet 50  $\mu\text{L}$  of the conjugate into each well except the Blank wells.
6. Pipet 50  $\mu\text{L}$  of the antibody into each well except the Blank and NSB wells.
7. Seal the plate. Incubate for 2 hours on a plate shaker (~500 rpm\*) at room temperature.
8. Empty the contents of the wells and wash by adding 400  $\mu\text{L}$  of wash buffer to every well. Repeat 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
9. Pipet 200  $\mu\text{L}$  of the streptavidin-HRP conjugate to each well except the Blank.
10. Seal the plate. Incubate for 30 minutes on a plate shaker (~500 rpm\*) at room temperature.
11. Wash as above (Step 8).
12. Add 200  $\mu\text{L}$  of the substrate solution into each well.
13. Incubate for 30 minutes at room temperature without shaking.
14. Pipet 50  $\mu\text{L}$  of the stop solution into each well.
15. After blanking the plate reader against the substrate blank, read optical density at 450 nm. If plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

\* The plate shaker speed was based on a BellCo Mini Orbital Shaker (mod no. 7744-08096). The actual speed of the plate shaker should be such that the liquid in the plate wells mixes thoroughly, but does not splash out of the well.





Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

## Calculation of Results

Several options are available for the calculation of the concentration of Bradykinin in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic (4PL) curve fitting program. Assay Blaster! assay analysis software (Cat. #ADI-28-0002) is an easy-to-use and cost effective program that provides the options of point-to-point, 4PL and 5PL curve fitting options.

The concentration of Bradykinin can be calculated as follows.

1. Calculate the average net OD for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

$$\text{Average Net OD} = \text{Average OD} - \text{Average NSB OD}$$

2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

$$\text{Percent Bound} = \frac{\text{Net OD}}{\text{Net Bo OD}} \times 100$$

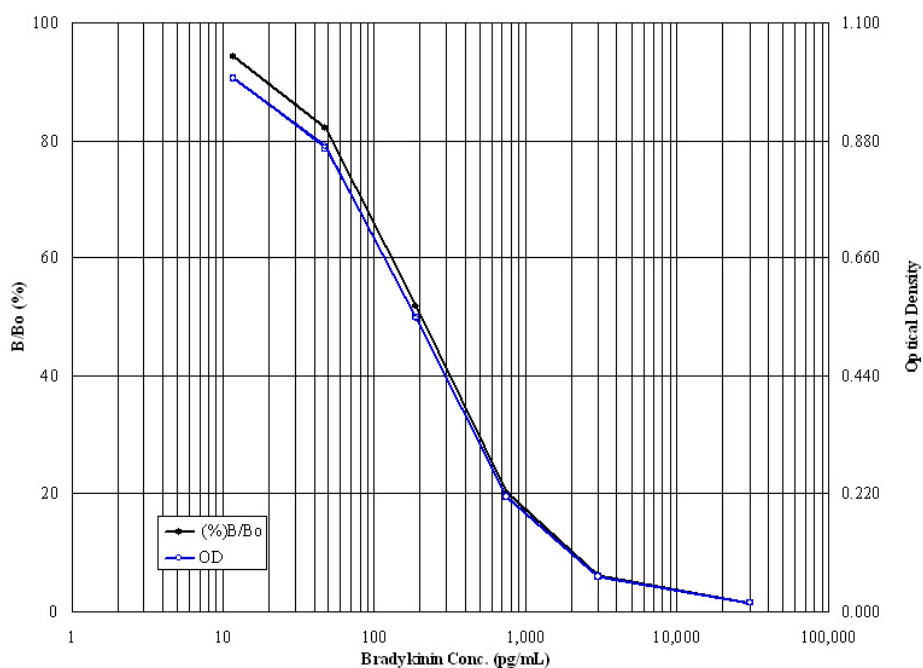
3. Plot the Percent Bound (B/Bo) versus concentration of Bradykinin for the standards. Approximate a straight line through the points. The concentration of Bradykinin of the unknowns can be determined by interpolation.

Samples with concentrations outside of the standard curve range will need to be re-analyzed using a different dilution.

## Typical Results

The results shown below are for illustration only and should not be used to calculate results from another assay.

| Sample         | Average Net OD | Percent Bound | Bradykinin (pg/mL) |
|----------------|----------------|---------------|--------------------|
| Blank (mean)   | (0.04)         | ---           | ---                |
| NSB            | 0.004          | 0%            | ---                |
| B <sub>0</sub> | 1.057          | 100%          | 0.0                |
| S1             | 0.016          | 1.5%          | 30000.0            |
| S2             | 0.065          | 6.2%          | 3000.0             |
| S3             | 0.215          | 20.3%         | 750.0              |
| S4             | 0.548          | 51.9%         | 187.5              |
| S5             | 0.869          | 82.3%         | 46.9               |
| S6             | 0.996          | 94.2%         | 11.7               |
| Unknown 1      | 0.244          | 23.0          | 637.6              |
| Unknown 2      | 0.78           | 73.8          | 75.5               |



## Typical Quality Control Parameters

Quality of Fit = 1.0000 (Calculated from 4 parameter logistic curve fit)

20% Intercept = 759 pg/mL

50% Intercept = 202 pg/mL

80% Intercept = 54 pg/mL

## Performance Characteristics

### Specificity

The cross reactivities for a number of related compounds were determined by diluting the cross reactants to concentrations in the range of 0.1 pM to 500 nM. These samples were then measured in the assay.

| Analyte                          | Sequence   | Percent cross-reactivities in the range of 0.1 pM - 500 nM |
|----------------------------------|------------|--|
| Bradykinin                       | RPPGFSPFR  | 100%   |
| Lys-Bradykinin (Kallidin)        | KRPPGFSPFR | 100%   |
| Les-Des-Arg9-Bradykinin          | KRPPGFSPF  | <1%  |
| BK1-5 stable degradation product | RPPGF      | <0.1%  |

### Sensitivity

The sensitivity, defined as 2 standard deviations from the mean signal at zero, was determined from 12 independent standard curves. The standard deviation was determined from 12 zero standard replicates. The sensitivity of the assay was determined to be 24.8 pg/mL.

### Parallelism and Dilutional Linearity

Human samples containing Bradykinin were serially diluted 1:4 in the kit assay buffer and measured in the assay. The results are shown in the table below.

| Dilution | Average % of Expected |       |       |
|----------|-----------------------|-------|-------|
|          | Plasma                | Serum | Urine |
| Neat     | ---                   | ---   | ---   |
| 1:4      | 93%                   | 111%  | ---   |
| 1:16     | 107%                  | 108%  | 100%  |
| 1:64     | ---                   | 100%  | ---   |
| 1:256    | ---                   | ---   | ---   |

### Precision

Intra-assay precision was determined by assaying 20 replicates of 3 buffer controls containing Bradykinin in a single assay.

| pg/mL | %CV |
|-------|-----|
| 695.4 | 4.6 |
| 208.7 | 6.2 |
| 73.7  | 9.9 |

Inter-assay precision was determined by measuring buffer controls of varying Bradykinin concentrations in multiple assays over several days.

| pg/mL | %CV  |
|-------|------|
| 700.4 | 15.0 |
| 209.3 | 10.3 |
| 66.1  | 11.9 |

## References

1. Bradykinin, a hypotensive and smooth muscle stimulating factor released from plasma globulin by snake venoms and by trypsin. Rocha E Silva M, Beraldo WT, Rosenfeld G. *Am J Physiol.* 1949 Feb;156(2):261-73.
2. Les substances hypotensives de l'urine humaine normale. Abelous JE and Bardier E. *CR Soc Biol* 66: 511–520, 1909.
3. The kallikrein-kinin system: current and future pharmacological targets. Moreau ME, Garbacki N, Molinaro G, Brown NJ, Marceau F, Adam A. *J Pharmacol Sci.* 2005 Sep;99(1):6-38.
4. Metabolism of bradykinin In vivo in humans: identification of BK1-5 as a stable plasma peptide metabolite. Murphey LJ, Hachey DL, Oates JA, Morrow JD, Brown NJ. *J Pharmacol Exp Ther.* 2000 Jul;294(1):263-9.
5. Kinins in humans. Duncan AM, Kladis A, Jennings GL, Dart AM, Esler M, Campbell DJ. *Am J Physiol Regul Integr Comp Physiol.* 2000 Apr;278(4):R897-904.

## Notes

Notes



#### USE FOR RESEARCH PURPOSES ONLY

Unless otherwise specified expressly on the packaging, all products sold hereunder are intended for and may be used for research purposes only and may not be used for food, drug, cosmetic or household use or for the diagnosis or treatment of human beings. Purchase does not include any right or license to use, develop or otherwise exploit these products commercially. Any commercial use, development or exploitation of these products or development using these products without the express written authorization of Enzo Life Sciences, Inc. is strictly prohibited. Buyer assumes all risk and liability for the use and/or results obtained by the use of the products covered by this invoice whether used singularly or in combination with other products.

#### LIMITED WARRANTY; DISCLAIMER OF WARRANTIES

These products are offered under a limited warranty. The products are guaranteed to meet all appropriate specifications described in the package insert at the time of shipment. Enzo Life Sciences' sole obligation is to replace the product to the extent of the purchasing price. All claims must be made to Enzo Life Sciences, Inc., within five (5) days of receipt of order. THIS WARRANTY IS EXPRESSLY IN LIEU OF ANY OTHER WARRANTIES OR LIABILITIES, EXPRESS OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NONINFRINGEMENT OF THE PATENT OR OTHER INTELLECTUAL PROPERTY RIGHTS OF OTHERS, AND ALL SUCH WARRANTIES (AND ANY OTHER WARRANTIES IMPLIED BY LAW) ARE EXPRESSLY DISCLAIMED.

#### TRADEMARKS AND PATENTS

Enzo and BiScore are trademarks of Enzo Life Sciences, Inc. QIAquick and QIAamp are trademarks of QIAGEN GmbH. Several of Enzo's products and product applications are covered by US and foreign patents and patents pending.

**[www.enzolifesciences.com](http://www.enzolifesciences.com)**  
*Enabling Discovery in Life Science®*

#### Global Headquarters

**Enzo Life Sciences Inc.**  
10 Executive Blvd  
Farmingdale, NY 11735  
(p) 1-800-942-0430  
(f) 1-631-694-7501  
(e) [info-usa@enzolifesciences.com](mailto:info-usa@enzolifesciences.com)

#### Enzo Life Sciences (ELS) AG

Industriestrasse 17, Postfach  
CH-4415 Lause / Switzerland  
(p) +41/0 61 926 89 89  
(f) +41/0 61 926 89 79  
(e) [info-ch@enzolifesciences.com](mailto:info-ch@enzolifesciences.com)

Please visit our website at [www.enzolifesciences.com](http://www.enzolifesciences.com) for additional contact information.

**Catalog Number:** 25-0738  
**Copyright:** 2010

**Revision:** 06/07/2019