Product Manual

AMP'D® GLP-1 ELISA kit

Catalog #: ENZ-KIT104

96 Well Kit

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 NON-INFRINGEMENT 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

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending. Enzo is a trademark of Enzo Life Sciences, Inc.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

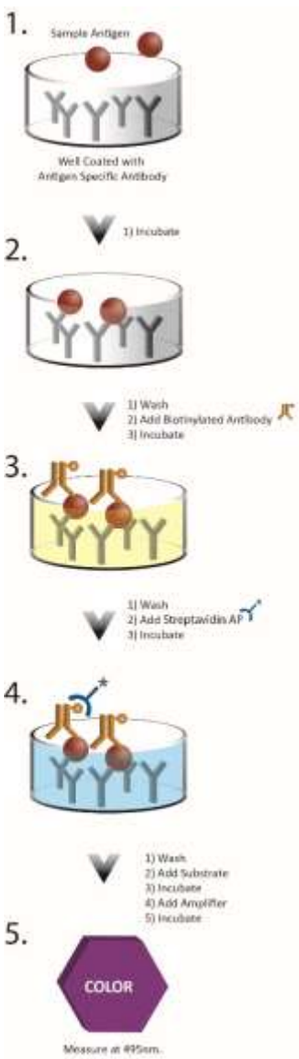
Product Manual

TABLE OF CONTENTS

Please read entire booklet before proceeding with the assay.

All reagents, except standard, should be stored at 4°C. Store standard at -20°C.

Please contact Enzo Life Sciences Technical Support if necessary.



INTRODUCTION

The AMP'D® GLP-1 ELISA kit is a complete kit for the quantitative determination of Glucagon-like peptide 1 (GLP-1) in serum and plasma samples of human origin. This kit is specific for the GLP-1 (7-36) amide and does not detect related molecules such as GLP-1 (1-36) amide, (9-36) amide, (7-37), GLP-2 and Glucagon. Please read the entire kit insert before performing this assay.

GLP-1, which is derived from proglucagon[1], is the most potent promoter of insulin secretion in the body[2] and a major incretin hormone.[3] It is secreted in response to oral ingestion of nutrients, with lipids and simple carbohydrates being the most potent stimulators of secretion.[4] GLP-1 is an important target in Type 2 diabetes, as it's secretion is diminished in these patients. The positive influence of GLP-1 on the metabolic disturbances of Type 2 diabetes, including stimulation of insulin secretion and inhibition of glucagon secretion, hepatic glucose production, gastric emptying and appetite, has provided a rationale for its therapeutic use in Type 2 diabetes.[5]

PRINCIPLE

1. Samples or standards are added to wells coated with a monoclonal antibody specific for GLP-1 (7-36) amide. The plate is then incubated.
2. The plate is washed, leaving only bound GLP-1 (7-36) amide on the plate. A yellow solution of a biotinylated monoclonal antibody, specific for GLP-1 (7-36, 7-37), is then added which will bind to the GLP-1 (7-36) amide captured on the plate. The plate is then incubated.
3. The plate is washed to remove excess antibody. A blue solution of Streptavidin conjugated Alkaline Phosphatase (SA-AP) is added to each well, which will bind to the biotinylated monoclonal antibody. The plate is again incubated.
4. The plate is washed to remove excess SA-AP conjugate. The AMP'D Substrate solution is then added to the wells and the plate is incubated.
5. Next, the AMP'D Amplifier solution is added to the wells and the plate incubated again.
6. Stop solution is added to stop the substrate reaction. The resulting purple color is read at 495nm. The amount of signal is directly proportional to the level of GLP-1 (7-36) amide in the sample.

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

MATERIALS SUPPLIED

1. **GLP-1 Microtiter Plate**
   One Plate of 96 Wells, Catalog No. 80-2647
   A plate using break-apart strips coated with a mouse monoclonal antibody specific to GLP-1 (7-36, amide).

2. **GLP-1 Detector Antibody**
   10 mL, Catalog No. 80-2648
   A yellow solution of a biotinylated mouse monoclonal antibody specific to GLP-1 (7-36, 7-37).

3. **Assay Buffer 13**
   50 mL, Catalog No. 80-1500
   Tris buffered saline containing proteins and detergents.

4. **GLP-1 Conjugate**
   10 mL, Catalog No. 80-2649
   A blue solution of Streptavidin-conjugated Alkaline Phosphatase.

5. **Wash Buffer Concentrate**
   100 mL, Catalog No. 80-1287
   Tris buffered saline containing detergents.

6. **GLP-1 Standard**
   100 µL, Catalog No. 80-2646
   One vial containing 25 ng/ml GLP-1 (7-36) amide.

7. **AMP'D Substrate**
   Lyophilized, Catalog No. 80-2596
   Lyophilized Signal Amplification Substrate containing NADPH.

8. **AMP'D Amplifier**
   Lyophilized, Catalog No. 80-2598
   Lyophilized Signal Amplification Amplifier containing alcohol dehydrogenase and diaphorase.

9. **AMP'D Substrate diluent**
   5.5 mL, Catalog No. 80-2597
   A 1x diluent, containing preservatives, used to reconstitute lyophilized substrate.

10. **AMP'D Amplifier diluent**
    5.5 mL, Catalog No. 80-2599
    A 1x diluent, containing preservatives, used to reconstitute lyophilized amplifier.

11. **AMP'D Stop Solution**
    5 mL, Catalog No. 80-2601
    A 0.3M solution of sulfuric acid

12. **AMP'D GLP-1 Assay Layout Sheet**
    1 each, Catalog No. 30-0322.

13. **Plate Sealer**
    3 each, Catalog No. 30-0012

STORAGE

All components of this kit except the standard are stable at 4˚C. The standard must be stored at or below -20˚C. Shipping conditions may not reflect long term storage conditions.

Once reconstituted, the signal amplification substrate is stable at 4˚C for 1 week, and the amplifier is stable at -20˚C for 1 week.

OTHER MATERIALS NEEDED

1. Deionized or distilled water.
2. Precision pipets for volumes between 5 µL and 1,000 µL.
3. Repeater pipet for dispensing 50 µL and 100 µL.
4. Disposable beakers for diluting buffer concentrates.
5. Graduated cylinders.
6. A microplate shaker.
7. Adsorbent paper for blotting.
8. Microplate reader capable of reading at 495 nm.
9. Software (such as AssayBlaster™ catalog number ADI-28-0002) for extrapolating sample values from optical density readings utilizing a four parameter logistic curve fit.

SAMPLE HANDLING

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

This assay is suitable for measuring GLP-1 (7-36) amide in serum and plasma. EDTA, Sodium Citrate and Sodium Heparin plasma were all validated for use. Prior to sample analysis in the assay, frozen samples should be slowly thawed at room temperature and vortexed to mix.

Plasma and Serum Preparation

1. **For plasma**, collect whole blood using the **BD™ P700** Blood Collection and Preservation system (Cat. no.366473), which contains both EDTA and DPP-4 protease inhibitor. DPP-4 is necessary for the preservation of the endogenous GLP-1.
2. An alternative to the BD™ P700 tubes would be the lavender cap Vacutainer® EDTA-plasma tube. To inhibit GLP-1 breakdown by DPP-4 protease add 100 µM Diprotin A (ALX-260-036) or 1 mM P32/98 (BML-PI142) to all samples immediately after blood drawing.
3. **For serum**, collect blood in serum collection tubes and allow to clot for 30 minutes. *This is not necessary for plasma samples.*
4. For both sample types, centrifuge at 1000 x g for 15 minutes at 4°C.
5. Place supernatants in a clean tube.
6. The supernatants may be dispensed into aliquots and stored at or below -20°C, or used immediately in the assay.

Samples must be stored at or below -20°C to avoid loss of bioactive analyte. Avoid repeated freeze/ thaw cycles.

For serum and plasma, the minimal dilution required will vary for different samples. Through internal testing it has been determined that a 1:2 or 1:4 dilution in assay buffer, depending on the matrix tested, will remove sample matrix interference in the assay (please refer to the Spike and Recovery section on page 6 for detailed data). However, due to variation in samples, a different dilution may be required. Users must determine the optimal dilutions for their particular experiments.

The following experiments with human serum and plasma were utilized to determine the final recommended sample dilutions.

SAMPLE MATRIX PROPERTIES

Linearity

Human serum or plasma was diluted 1:2 in assay buffer and spiked with human GLP-1 (7-36) amide to 200 pg/mL. The spiked and diluted samples were then serially diluted 1:2 in assay buffer and compared to the standard curve. The results are shown in the table below.

		Dilutional linearity, %			
GLP-1 pg/mL	**Dilution**	**Serum**	**EDTA Plasma**	**Na Citrate Plasma**	**Na Heparin Plasma**
200	1:2	68.2	109	51.5	53.6
100	1:4	80.7	86.4	136.4	81.8
50	1:8	90	81	93.4	76.5
25	1:16	100	100	100	100

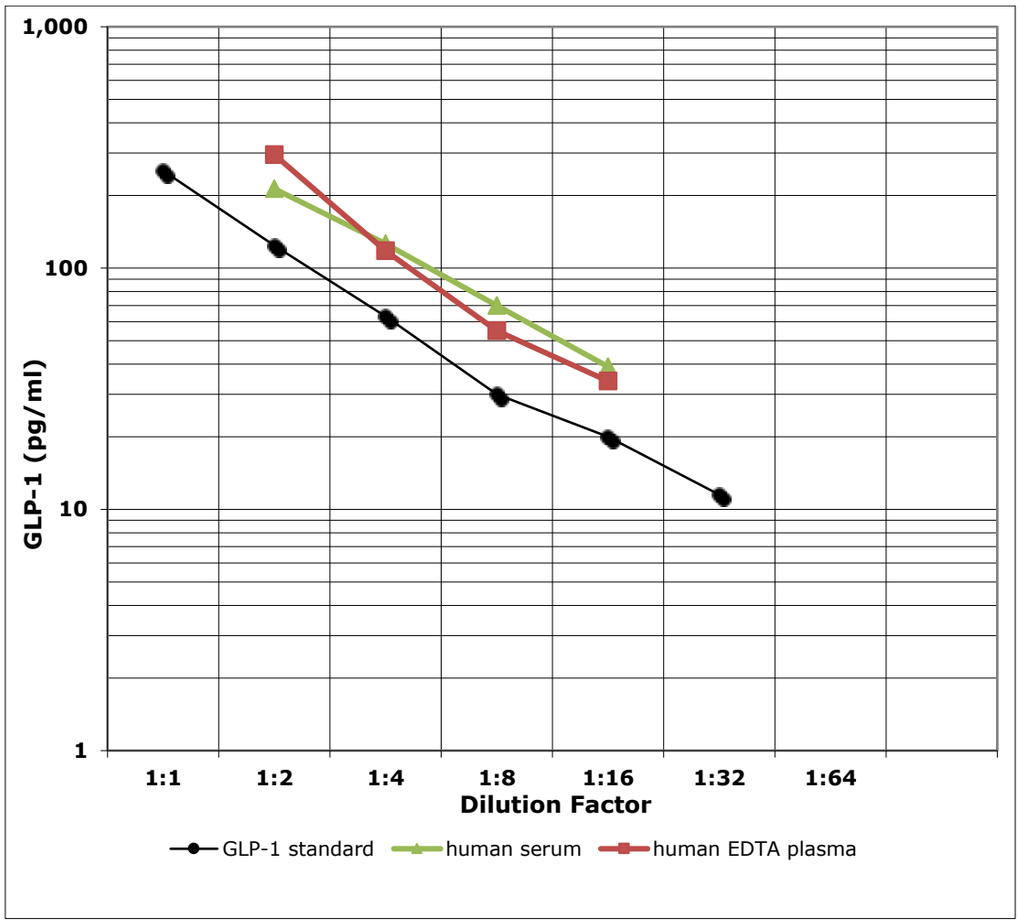
Spike and Recovery

GLP-1 (7-36) amide was spiked at 100 pg/mL into diluted human serum or plasma. Matrix background was subtracted and the recovery was compared to the recovery of identical GLP-1 (7-36) amide spiked into assay buffer. The average percent recovery for each matrix at the minimum recommended dilution is indicated below.

Sample	% Recovery	Minimum Recommended Dilution
Serum	88.6	1:2
EDTA Plasma	88.6	1:2
Na Citrate Plasma	99.5	1:4
Na Heparin Plasma	114.7	1:4

Parallelism

To assess parallelism, human serum and EDTA plasma was diluted to 1:2, spiked with GLP-1 (7-36) amide and then serially diluted into assay buffer and run in the assay. The GLP-1 concentration in each sample was assigned using the standard curve. Assigned concentrations were plotted as a function of sample dilution. Parallelism of the curves demonstrates that the antigen binding characteristics are similar enough to allow the accurate determination of native analyte levels in diluted samples of human origin.



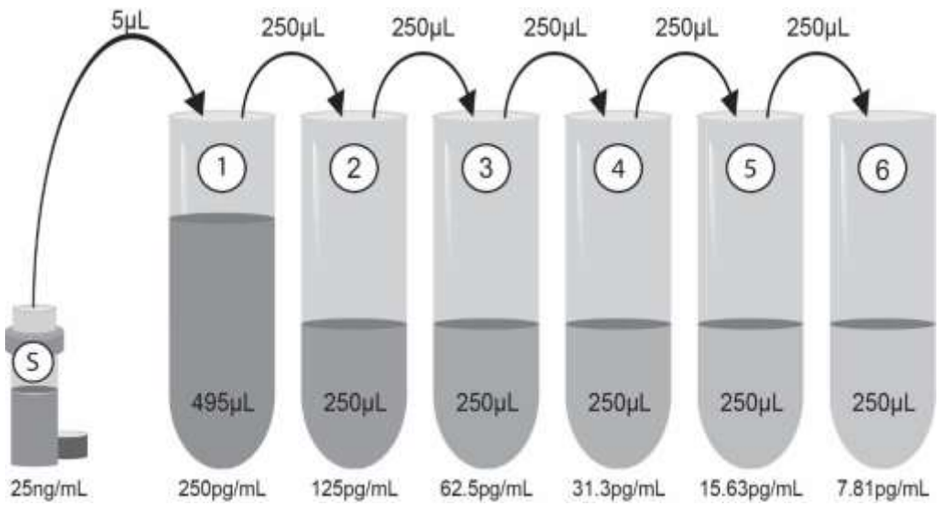
REAGENT PREPARATION

1. **Wash Buffer**

   Prepare the Wash Buffer by diluting 50 mL of the supplied concentrate with 950 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. **GLP-1 Standard Curve**

   The GLP-1 standard stock as well as diluted standards and samples should be kept on ice and used within 60 minutes of preparation for optimal performance. Before adding diluted standards to the microplate, allow them to briefly equilibrate to room temperature.



   Label six 12x75 mm polypropylene tubes #1 through #6. Pipet 495 µL of Assay Buffer 13 into tube #1. Pipet 250 µL of Assay Buffer 13 into tube #2 through tube #6. Add 5 µL of 25 ng/mL GLP-1 standard stock to tube #1 and vortex. Add 250 µL of tube #1 into tube #2 and vortex. Add 250 µL of tube #2 to tube #3 and vortex thoroughly. Continue this for tubes #4 through #6.

   Diluted standards should not be stored for re-use. Make new standard preparations with each use.

3. **Reconstituting Substrate**

   Reconstitute the substrate 10-20 minutes before use by adding 5.5 mL of 1x substrate diluent directly to the substrate vial. Gently mix until dissolved. Store any remaining reconstituted substrate at 4°C where it is stable for 1 week.

4. **Reconstituting Amplifier**

   Reconstitute the amplifier 10 minutes before use by adding 5.5 mL of 1x amplifier diluent directly to the amplifier vial.

Gently mix until dissolved. Store any remaining reconstituted substrate at -20°C where it is stable for 1 week.

ASSAY PROCEDURE

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove unneeded wells and return them, with the desiccant, to the plate bag and seal. Store the unused wells at 4°C.

1. Pipet 100 µL of that assay buffer into the S0 (0pg/mL standard) wells.
2. Pipet 100 µL of Standards #1 through #6 into the appropriate wells.
3. Pipet 100 µL of the Samples into the appropriate wells.
4. Seal the plate and incubate at room temperature (RT) on a plate shaker for 1 hour at ~500rpm*.
5. Empty the contents of the wells and wash by adding 300 µL of wash solution to every well. Repeat the wash 2 more times for a total of 3 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.
6. Pipet 100 µL of yellow Antibody into each well except the blank.
7. Seal the plate and incubate at RT on a plate shaker for 1 hour at ~500rpm*.
8. Wash as above (Step 5).
9. Add 100 µL of blue Conjugate to each well, except the Blank.
10. Seal the plate and incubate at RT on a plate shaker for 30 minutes at ~500rpm*.
11. Wash as above (Step 5).
12. Pipet 50 µL of Substrate solution into each well. Incubate for 15 minutes at RT on a plate shaker at ~500rpm*. **See note on following page.**
13. Pipet 50 µL Amplifier solution to each well. Incubate for 15 minutes at RT on a plate shaker at ~500rpm*. **See note on following page.**
14. Pipet 50 µL of the stop solution into each well.
15. After zeroing the plate reader against signal amplification blank, read optical density at 495nm. If the plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the signal amplification blank from all readings.

Note: It is important to add the substrate and amplifier to the wells in the same sequence. Strict control of reagent delivery and incubation times should be adhered to for the most accurate and reproducible results between experimental replicates/runs.

*** 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.**

CALCULATION OF RESULTS

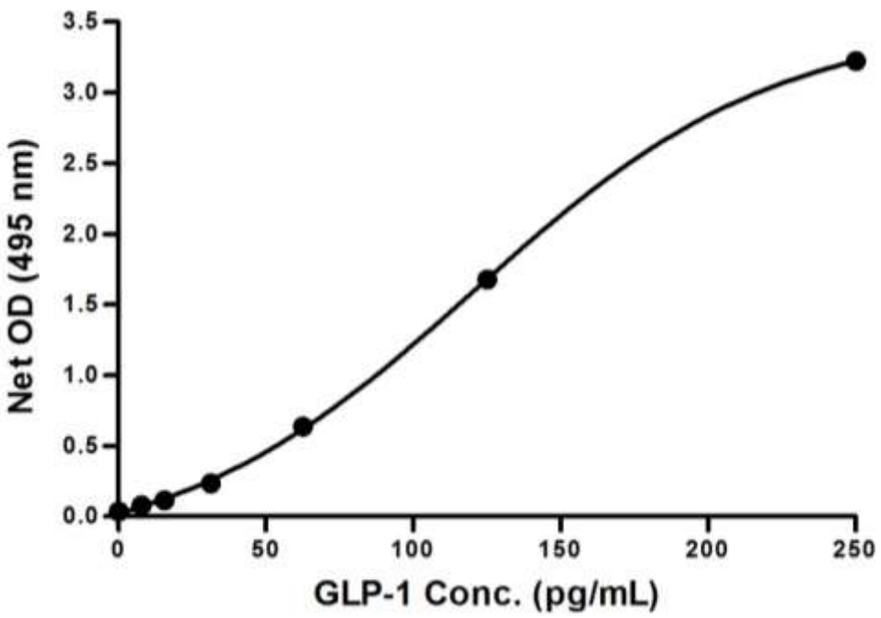
Several options are available for the calculation of the concentration of GLP-1 (7-36) amide in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic (4PL) curve fitting program. Assay Blaster! Data analysis software (Prod. no. ADI-28-0002) 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 GLP-1 (7-36) amide can be calculated as follows:

1. Calculate the average OD for each standard and sample.
2. Using data analysis software, plot the Average OD for each standard versus GLP-1 (7-36) amide concentration in each standard.

TYPICAL RESULTS

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

Sample	Optical Density (495nm)	GLP-1 (pg/mL)
S0	0.035	**0**
S1	3.224	**250**
S2	1.678	**125**
S3	0.637	**62.5**
S4	0.234	**31.3**
S5	0.116	**15.6**
S6	0.079	**7.8**



PERFORMANCE CHARACTERISTICS

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[6].

Specificity

The specificity of the assay was determined by diluting the cross reactant in the kit assay buffer at a concentration of ten times the high standard and then measuring in the assay.

Analyte	Cross Reactivity
GLP-1 (7-36) amide	100%
GLP-1 (9-36) amide	≤0.02%
GLP-1 (1-36) amide	≤0.02%
GLP-1 (7-37)	≤2.1%
GLP-2	≤0.02%
Glucagon	≤0.02%

Sensitivity

The sensitivity or limit of detection of the assay is 5.525 pg/mL, determined by interpolation at 2 standard deviations away from the mean signal of 50 replicates of 0 pg/mL. Data was used from 4 standard curves.

Interference

Protease inhibitors commonly used in clinical specimens were analyzed for interference in the assay and the tolerance was determined.

Protease inhibitor	Assay Tolerance
PIC	≤1.25%
PMSF	≤1mM
Aprotinin	≤100 ug/mL

Intra-assay precision was determined by assaying 20 replicates of three buffer controls containing GLP-1 in a single assay.

Intra-assay precision	
pg/mL	**%CV**
95.8	10.3
45.1	14.0
35.9	13.6

Inter-assay precision was determined by measuring buffer controls of varying GLP-1 concentrations in multiple assays over several days.

Inter-assay precision	
pg/mL	**%CV**
95.3	10.6
45.2	20.2
28.7	17.8

REFERENCES

1. J. Schirra, et al., Gut, (2002) 50: 341-348.
2. W. Chai, et al., Diabetes, (2012) 61: 888-896.
3. M. Shigeto, et al., Journal of Pharmacological Sciences, (2008) 108: 274-279.
4. FP Knop, et al., American Journal of Physiology, Endocrinology and Metabolism, (2006) 292: E324-E330.
5. M-B Toft-Nielsen, et al., The Journal of Clinical Endocrinology and Metabolism, (2001) 86(8): 3717-3723.
6. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

GLOBAL HEADQUARTERS
Enzo Life Sciences Inc.
10 Executive Boulevard
Farmingdale, NY 11735
Toll-Free:1.800.942.0430
Phone:631.694.7070
Fax: 631.694.7501
info-usa@enzolifesciences.com

EUROPE/ASIA
Enzo Life Sciences (ELS) AG
Industriestrasse 17
CH-4415 Lausen
Switzerland
Phone:+41/0 61 926 89 89
Fax:+41/0 61 926 89 79
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:
www.enzolifesciences.com