Product Manual

# VEGF (human) ELISA Kit

Catalog #: ENZ-KIT156-0001

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.

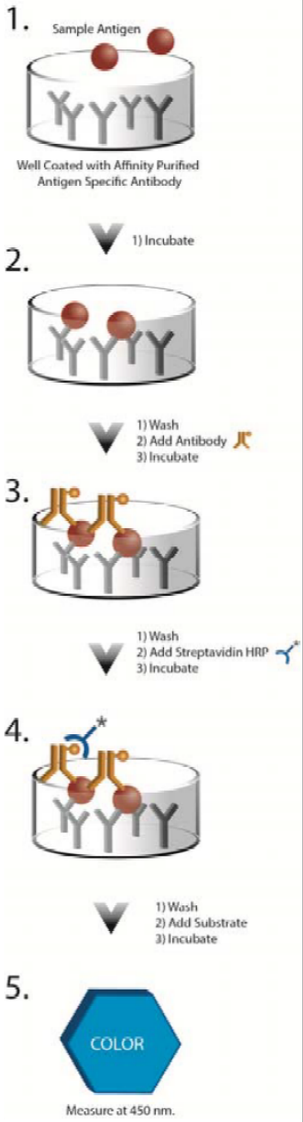
Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

## INTRODUCTION

The VEGF (human) ELISA kit is a complete kit for the quantitative determination of human VEGF in biological matrices. Please read the complete kit insert carefully before performing the assay.

Vascular Endothelial Growth Factor (VEGF), originally known as Vascular Permeability Factor (VPF), is a family of proteins that stimulate vasculogenesis and angiogenesis. The VEGF family is comprised of five members: VEGF-A, VEGF-B, VEGF-C, VEGF-D and PGF (Placenta Growth Factor)[1-3]. VEGF-A was first identified and is also referred to as just VEGF. Alternative splicing of an 8-exon VEGF mRNA results in multiple isoforms of 121, 145, 165, 183, 189, and 206 amino acids[3]. VEGF165 and VEGF121 are predominant isoforms expressed in most cells and tissues. VEGF165 is a 45 KDa homodimeric glycoprotein and has moderate affinity to heparin, which results in the mild interaction with extracellular matrix and balanced diffusion capability[4]. In contrast, VEGF 121 is a fully diffusive protein due to the lack of affinity to heparin[4]. VEGF specifically binds tyrosine kinase receptors VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1) to activate downstream signaling pathways leading to endothelial cell survival, proliferation, migration, and vascular permeability[5]. VEGF plays essential role in normal vascular embryonic and reproductive angiogenesis. On the other hand, VEGF is central to pathological angiogenesis by enhancing vessel permeability or recruiting endothelial progenitor cells from long distances. The understanding of VEGF in promoting pathological angiogenesis and tumor metastasis has led to the rational studies on blocking the VEGF/VEGFR axis in inhibiting angiogenesis and tumor growth in multiple clinical models[6-9].




## PRINCIPLE

1. A monoclonal antibody to VEGF is immobilized on a microtiter plate. Standards and samples containing VEGF are added to the plate and incubated.
2. During this incubation the VEGF antibody immobilized on the plate binds VEGF in the standards or samples.
3. The plate is washed, removing excess unbound sample or standard, and a solution of biotinylated detector antibody to VEGF is added. This antibody binds to the VEGF captured on the plate.
4. After a short incubation the plate is washed to remove unbound detector antibody. Streptavidin conjugated to Horseradish Peroxidase (SA-HRP) is added to bind the biotinylated VEGF detector antibody. The plate is then incubated.
5. The plate is washed to remove excess conjugate. TMB substrate solution is added to all wells and incubated. An HRP-catalyzed reaction generates a blue color in the solution.
6. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450nm. The amount of signal is directly proportional to the level of VEGF in the sample or standard.

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

## MATERIALS SUPPLIED

1. **VEGF Microtiter Plate, One plate of 96 wells**
   **Catalog No. 80-2744**

   Plate with break-apart strips coated with a mouse monoclonal antibody specific to VEGF.

2. **VEGF Standard, 100µl**
   **Catalog No. 80-2746**

   One vial containing 100µl of 60,000pg/ml recombinant VEGF protein.

3. **VEGF Detector Antibody 100X, 120µl**
   **Catalog No. 80-2748-0120**

   One vial containing 120µl of 100X concentrated biotinylated VEGF detector antibody.

4. **VEGF Conjugate, 11ml**
   **Catalog No. 80-2745-0011**

   A blue solution of streptavidin conjugated to horseradish peroxidase.

5. **Antibody Diluent, 12ml**
   **Catalog No. 80-2747-0012**

   A yellow solution for dilution of VEGF detector antibody.

6. **Assay Buffer 10, 50ml**
   **Catalog No. 80-2735-0050**

   Tris buffered saline containing detergents.

Protect substrate from prolonged exposure to light.

7. **TMB Substrate, 10ml**
   **Catalog No. 80-0350**

   Solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide. Protect from prolonged exposure to light.

HCl is caustic. Keep tightly capped.

8. **Stop Solution 2, 10ml**
   **Catalog No. 80-0377**

   A 1N solution of hydrochloric acid in water. Keep tightly capped. Caution: **Caustic**.

9. **Wash Buffer Concentrate, 100ml**
   **Catalog No. 80-1287**

   20x Tris buffered saline containing detergents.

10. **VEGF Assay Layout Sheet, 1 each**
    **Catalog No. 30-0342.**

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

## STORAGE

All components of this kit should be stored at 4°C until the kit's expiration date.

## OTHER MATERIALS NEEDED

1. Deionized or distilled water.
2. Precision pipets for volumes between 5µL and 1,000µL.
3. Repeater pipet for dispensing 100µL.
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 450 nm.
9. Software for extrapolating sample values from optical density readings utilizing a four parameter logistic curve fit.

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

## SAMPLE HANDLING

This assay is suitable for the measurement of VEGF in human serum, plasma (EDTA) and tissue culture media. Prior to assay, frozen specimens should be brought to 4°C and centrifuged. If it is necessary, filter to remove residual debris.

Diluted samples have been validated for use in this assay (please refer to the Spike and Recovery section on page 7 for details). However, due to variation in samples, users must determine the optimal dilutions for their unique set of samples.

## SAMPLE MATRIX PROPERTIES

### Linearity

The minimum required dilution for human serum and plasma (EDTA) was determined by serially diluting specimens into Assay Buffer 10 and identifying the dilution at which linearity was observed. According to the table below, the minimum required dilution in human serum is 1:8 and in human plasma is 1:2.

| Dilutional Linearity, % | | |
|---|---|---|
| **Dilution** | **Human Serum** | **Human Plasma** |
| **1:2** | --- | 100 |
| **1:4** | --- | 114 |
| **1:8** | 100 | 122 |
| **1:16** | 102 | --- |
| **1:32** | 109 | --- |
| **1:64** | 114 | --- |
| **1:128** | 111 | --- |

### Spike and Recovery

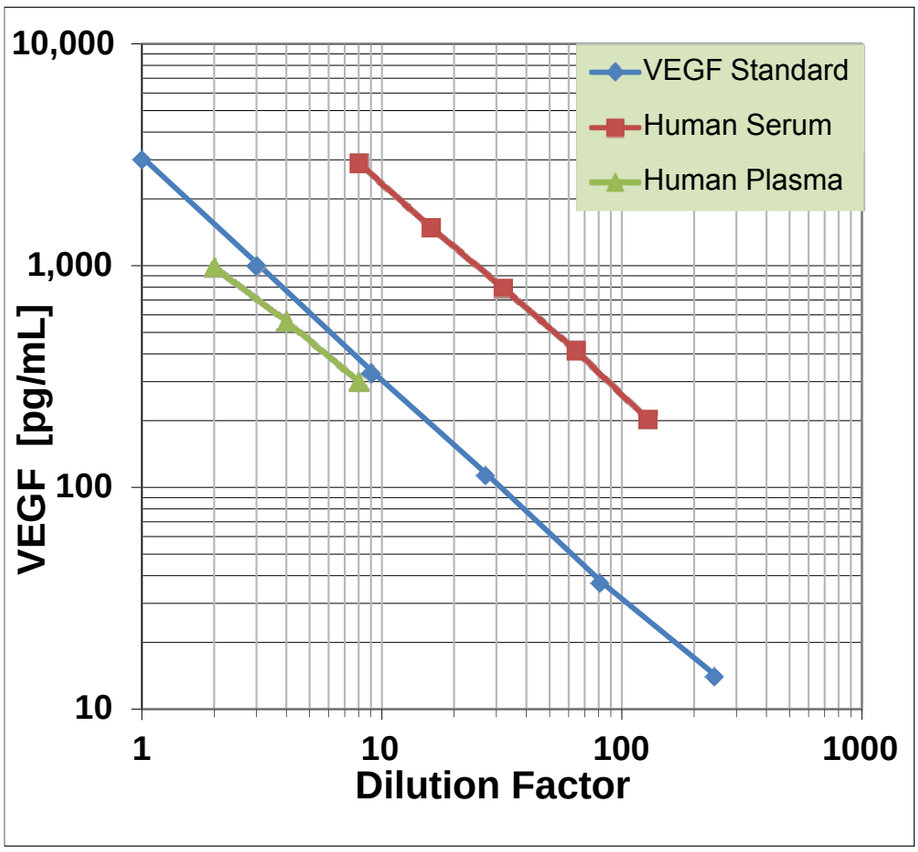
Purified recombinant human VEGF 165 protein was spiked at 1000, 400, and 16 pg/ml of concentrations into respectively diluted human matrices. Matrix background was subtracted from the spiked values and the average percent recovery for each matrix at indicated dilution is presented below. These results showed the tested human matrices and tissue culture media at recommended dilution have no obvious interference with the VEGF assay.

| Sample | Spike Concentration (pg/ml) | % Recovery | Dilution |
|---|---|---|---|
| **Human Serum** | 1000 | 105 | 1:16 |
| | 400 | 104 | |
| | 16 | 99 | |
| **Human Plasma** | 1000 | 72 | 1:2 |
| | 400 | 78 | |
| | 16 | 95 | |
| ***Tissue Culture Medium** | 1000 | 105 | 1:2 |
| | 400 | 117 | |
| | 16 | 112 | |

*Tissue culture medium used in this test was DMEM with phenol red and 10% FBS.

**Parallelism**

Parallelism experiments were carried out to determine if the VEGF standard accurately mimicked native VEGF in biological matrices. Human serum or plasma was serially diluted into Assay Buffer 10 and run in the assay. The VEGF concentration in each sample was assigned using the standard curve. The parallel response indicates the VEGF standard effectively mimics the native protein.



Sample handling procedures should be completed prior to reagent preparation.

Polypropylene tubes may be used for standard preparation. Avoid polystyrene.

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

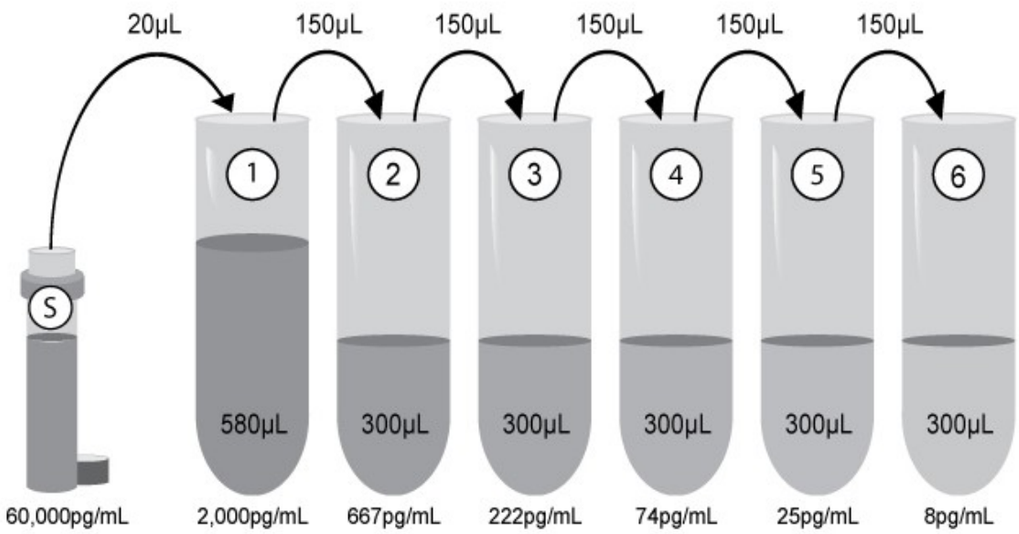
## REAGENT PREPARATION

### 1. Wash Buffer

Prepare the Wash Buffer by diluting 50mL of the supplied concentrate with 950mL of deionized water. The diluted wash buffer can be stored at room temperature for up to 3 months.

### 2. VEGF Standard Curve

Label six 12 x 75mm (or similar) polypropylene tubes #1 through #6. Add 580µl Assay Buffer 10 into tube #1 and 300µl Assay Buffer 10 into tubes #2 through #6. Add 20µL of 60,000pg/mL standard stock into tube #1 and vortex gently. Keep the concentrated standard stock on ice or at 4°C right away. Serially dilute 150µL of tube #1 standard to tubes #2 through #6 by gently vortexing after each serial dilution transfer. The diluted standards and samples should be kept on ice if a prolonged amount of bench set-up time is anticipated. Allow the VEGF standards and samples to warm to room temperature before adding to the plate.




**Diluted standards should be used within 30 minutes of preparation. Discard any unused standard dilutions.**

### 3. VEGF Detector Antibody

Dilute appropriate volume of 100X concentrated detector antibody by adding 10µL of stock solution into 990µL of Antibody Diluent and store the rest of 100X concentrated stock at 4°C. Discard any unused 1X diluted antibody.

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

All standards and samples should be run in duplicate.

Add the reagents to the sides of the wells to avoid possible contamination.

## 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. Add 100µL of Assay Buffer 10 into NSB (non-specific binding) and standard 0 wells. Leave the Blank and TA (total activity) wells empty.
2. Add 100µL of standards #1 through #6 into the appropriate wells.
3. Add 100µL of the samples into the appropriate wells.
4. Seal the plate and incubate at room temperature (RT) on a plate shaker for 60 min at ~500rpm*. **See note.**
5. Empty the contents of the wells and wash by adding a full well volume ~400µL of 1X Wash Buffer to each well. Empty or aspirate the wells and repeat the wash 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.
6. Add 100µL of diluted VEGF Detector Antibody into all wells except for the NSB, TA and blank. Add 100µL of Assay Buffer 10 to NSB wells and leave Blank and TA wells empty.
7. Seal the plate and incubate at RT on a plate shaker for 30 min at ~500rpm.
8. Wash as above (Step 5).
9. Add 100µL of VEGF Conjugate (blue) to each well, except the Blank and TA wells.
10. Seal the plate and incubate at RT on a plate shaker for 30 minutes at ~500rpm.
11. Wash as above (Step 5).
12. Dilute VEGF Conjugate 1:50 with Assay Buffer 10 and add 5µL of this dilution to TA wells.
13. Add 100µL TMB solution into all wells.
14. Seal the plate and incubate at RT on a plate shaker for 30 minutes at ~500rpm.
15. Add 100µL of the Stop Solution 2 into each well.
16. After blanking the plate reader against the substrate, read optical density at 450nm. If the plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

*** <u>Note:</u> 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.**

Be 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 VEGF in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic (4PL) curve fitting program. The concentration of VEGF 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.

   Average Net OD = Average OD - Average NSB OD

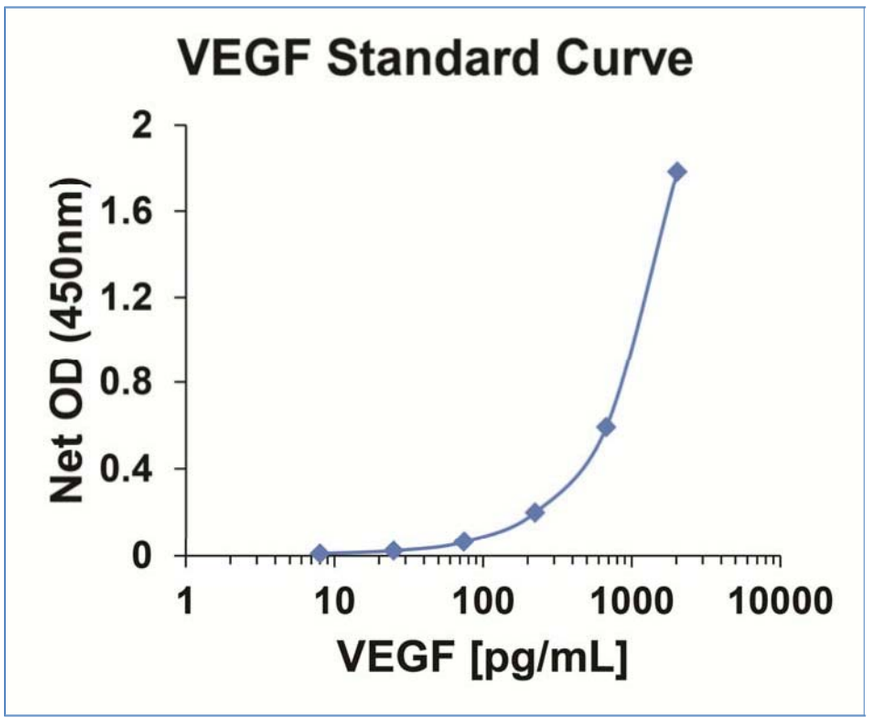
2. Using Data Analysis Software plot the Average Net OD versus concentration of VEGF for the standards utilizing a 4PL curve fit. The concentration of VEGF in the unknown samples can be determined by interpolation.

   Samples with concentrations outside of the standard curve range will need to be reanalyzed using alternative dilution.

## TYPICAL RESULTS

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

| Sample | Raw OD | Net OD | VEGF [pg/mL] |
|---|---|---|---|
| NSB | 0.048 | --- | --- |
| Std 0 | 0.052 | 0.003 | 0 |
| Std 1 | 1.835 | 1.786 | 2000 |
| Std 2 | 0.640 | 0.592 | 667 |
| Std 3 | 0.247 | 0.199 | 222 |
| Std 4 | 0.113 | 0.064 | 74 |
| Std 5 | 0.072 | 0.024 | 25 |
| Std 6 | 0.059 | 0.010 | 8 |



## PERFORMANCE CHARACTERISTICS

### Specificity

The cross reactivities for a number of related growth factors was determined by diluting the cross reactants in the kit assay buffer at a concentration of ten times the top standard. The concentration of these samples was then measured in the assay. The percentage of cross reactivity was calculated by comparing the detected concentration with input concentration as a percentage. The cross reactivity to these samples was ≤ 0.06%.

Species cross reactivity was tested in mouse or rat serum and plasma. The assay detected some background signals, but the signals could not be linearly diluted out. Spiking mouse or rat recombinant proteins into assay buffer or matrices, there was no spiked protein detected. Therefore, this assay may have some non-specific binding in mouse or rat serum, but it can't detect mouse or rat VEGF. This assay is a human VEGF specific assay.

| Analytes | Cross Reactivity |
|---|---|
| human VEGF 165 | 100% |
| human VEGF 121 | 0.06% |
| human PDGF-AA | 0.013% |
| human HGF | Undetected |
| human IL-10 | Undetected |
| human IL-8 | 0.007% |
| human GRO | 0.01% |
| human G-CSF | 0.023% |
| human SCF | Undetected |
| human Endothelin-1 | 0.003% |
| human Big Endothelin-1 | Undetected |
| human Endothelin-3 | Undetected |

**Sensitivity**

The sensitivity or limit of detection of this assay is 4.712pg/mL. The assay sensitivity was determined by interpolation at two standard deviations above the net OD of 20 zero standard replicates utilizing a four parameter logistic (4PL) curve fit.

**Intra-assay precision** was determined by analyzing 20 replicates of three matrix controls containing VEGF in a single assay.

| Intra-assay Precision | |
|---|---|
| [pg/mL] | %CV |
| 633.6 | 3.7 |
| 201.8 | 3.9 |
| 46.5 | 7.3 |

**Inter-assay precision** was determined by measuring matrix controls containing VEGF in multiple assays over several days.

| Inter-assay Precision | |
|---|---|
| [pg/mL] | %CV |
| 680.7 | 10.9 |
| 180.9 | 13.6 |
| 45.3 | 12.8 |

## REFERENCES


1. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. FASEB J.
2. 1999 Jan; 13(1):9-22
3. Bhisitkul RB. Br J Ophthalmol. 2006 Dec; 90(12):1542–1547.
4. Robinson CJ, Stringer SE. J Cell Sci. 2001 Mar; 114(Pt 5):853-65.
5. Stimpfl M, Tong D, Fasching B, Schuster E, et al. Clin Cancer
6. Res. 2002 Jul; 8(7):2253-9.
7. Giles FJ. Oncologist. 2001; 6 Suppl 5:32-9.
8. Carmeliet P, Jain RK. Nature. 2000 Sep 14; 407(6801):249-57.
9. Tímár J, Döme B, Fazekas K, Janovics A, Paku S. Pathol Oncol
10. Res. 2001;7(2):85-94.
11. Yadav L, Puri N, Rastogi V, Satpute P, Sharma V. J Clin Diagn
12. Res. 2015 Jun; 9(6):XE01-XE05.
13. Wise LM, Veikkola T, Mercer AA, Savory LJ, et al. Proc Natl
14. Acad Sci U S A. 1999 Mar 16;96(6):3071-6.

## NOTES

## NOTES

## NOTES

**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

Catalog Number: ENZ-KIT156

Rev. 11/03/2015