



**Human Resistin**

**96-Well Plate**

**Cat. # EZHR-95K**

**HUMAN RESISTIN ELISA KIT**  
**96-Well Plate (Cat. # EZHR-95K)**

I. Intended Use	2
II. Principles Of Procedure	2
III. Reagents Supplied	2
IV. Storage and Stability	3
V. Reagent Precautions	4
VI. Materials Required But Not Provided	6
VII. Sample Collection And Storage	6
VIII. Sample Preparation	7
IX. Standard and Quality Controls Preparation	7
X. Assay Procedure	8
XI. Microtiter Plate Arrangement	11
XII. Calculations	12
XIII. Assay Characteristics	12
XIV. Standard Curve	15
XV. Method Comparison	16
XVI. Quality Controls	17
XVII. Troubleshooting Guide	17
XVIII. References	18
XIX. Replacement Reagents	18
XX. Ordering Information	18

## I. INTENDED USE

This Human Resistin ELISA kit is used for the non-radioactive quantification of human resistin in serum, plasma and other biological media. One kit is sufficient to measure 38 unknown samples in duplicate. ***This kit is for Research Use Only. Not for Use in Diagnostic Procedures.***

## II. PRINCIPLES OF PROCEDURE

This assay is based, sequentially, on: 1) capture of human resistin from sample by a monoclonal antibody, immobilized in the wells of a microwell plate, 2) washing off unbound materials including free materials from samples, 3) binding of the biotinylated monoclonal human resistin antibody to the other side of captured human resistin molecules, 4) conjugation of SA-HRP (Poly-HRP-labeled streptavidin) enzyme to the biotinylated antibodies, and 5) quantification of bound detection conjugate by monitoring SA-HRP enzyme activity in the presence of TMB (tetramethylbenzidine) substrates. The enzyme activity is measured spectrophotometrically by the absorbency at 450 nm due to production of the photometric product. Since the amount of photometric product is directly proportional to the concentration of human resistin 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 human resistin.

## III. REAGENTS SUPPLIED

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

### A. Human Resistin ELISA Plate

Coated with anti- Human Resistin Monoclonal Antibody

Quantity: 1 plate

Preparation: Ready to use

### B. Adhesive Plate Sealer

Quantity: 2 Sheets

Preparation: Ready to use

### C. 10X HRP Wash Buffer Concentrate

10X concentrate of 50 mM TBS Buffer containing 0.05% Tween 20

Quantity: 2 bottles containing 50 mL each

Preparation: Dilute 1:10 with distilled or deionized water

### D. Human Resistin Standards

Human Resistin, lyophilized.

Quantity: 2.5 ng/0.25 mL (10 ng/mL) upon hydration.

Preparation: Contents Lyophilized. Reconstitute with 250  $\mu$ L distilled or deionized water to obtain 10.0 ng/mL.

### III. REAGENTS SUPPLIED (continued)

#### E. Human Resistin Quality Controls 1 and 2

One vial each, lyophilized, containing Human Resistin in Assay Buffer.

Quantity: 0.25 mL/vial upon hydration.

Preparation: Contents Lyophilized. Reconstitute each vial with 250  $\mu$ L distilled or deionized water.

#### F. Assay Buffer

0.05M PBS, pH 7.4, containing 0.025 M EDTA, 0.08% Sodium Azide and 1% BSA.

Quantity: 12 mL

Preparation: Ready to use

#### G. Human Resistin Detection Antibody

Biotinylated anti-human resistin monoclonal antibody

Quantity: 9 mL

Preparation: Ready to use

#### H. Enzyme Solution

Pre-titered Streptavidin-Horseradish Peroxidase Conjugate (SA-HRP)

Quantity: 12 mL

Preparation: Ready to use

#### I. Substrate (Light sensitive, avoid unnecessary exposure to light)

3, 3', 5, 5'-tetramethylbenzidine (TMB)

Quantity: 12 mL

Preparation: Ready to use

#### J. Stop Solution (Caution: Corrosive Solution)

Quantity: 12 mL

Preparation: Ready to use

### IV. 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 thaws 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.

## **V. REAGENT PRECAUTIONS**

### **A. Sodium Azide**








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.

### **B. Hydrochloric Acid**

Hydrochloric Acid is corrosive and can cause eye and skin burns. It is harmful if swallowed and can cause respiratory and digestive tract burns. Avoid contact with skin and eyes.

**Note: See Full Labels of Hazardous components on next page.**

**Full labels of hazardous components in this kit:**

Ingredient, Cat #		Full Label	
Human Resistin Detection Antibody	E1095		<b>Warning.</b> Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Human Resistin Quality Controls I & II	E6095-K	 	<b>Danger.</b> Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye 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.
Human Resistin Standard	E8095-K	 	<b>Danger.</b> Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye 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.
Stop Solution	ET-TMB		<b>Warning.</b> May be corrosive to metals.
10X HRP Wash Buffer Concentrate	EWB-HRP		<b>Warning.</b> May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.

## **VI. MATERIALS REQUIRED BUT NOT PROVIDED**

1. Pipette with tips, 10  $\mu\text{L}$  – 200  $\mu\text{L}$
2. Multi-channel pipette, 50  $\mu\text{L}$  – 300  $\mu\text{L}$
3. Reagent reservoirs
4. Vortex mixer
5. Refrigerator
6. Deionized water
7. Microtiter plate reader capable of reading absorbency at 450 nm
8. Microtiter plate shaker
9. Absorbent Paper or Cloth

## **VII. SAMPLE COLLECTION AND STORAGE**

1. To prepare serum samples, whole blood is directly drawn into a centrifuge tube that contains no anti-coagulant. Let blood clot at room temperature for 30 minutes. Promptly centrifuge the clotted blood at 2,000 to 3,000 x g for 15 minutes at  $4 \pm 2^\circ\text{C}$ . Transfer and store serum samples in separate tubes. Date and identify each sample. Use freshly prepared serum or aliquot and store samples at  $\leq -20^\circ\text{C}$  for later use. For long-term storage, keep at  $-70^\circ\text{C}$ . Avoid freeze/thaw cycles.
2. To prepare plasma samples, whole blood should be collected into centrifuge tubes containing enough  $\text{K}_3\text{EDTA}$  to achieve a final concentration of 1.735 mg/mL and centrifuged immediately after collection. Observe the same precautions in the preparation of serum samples.
3. If heparin is to be used as an anticoagulant, the effect on the assay outcome at the dose of heparin used should be pre-determined.
4. Avoid using samples with gross hemolysis or lipemia.

## VIII. SAMPLE PREPARATION

1. Allow all the reagents to come to room temperature.
2. Dilute serum or plasma samples 1:10 in Assay Buffer. Cellular extract and culture media dilutions will vary.

## IX. STANDARD AND QUALITY CONTROLS PREPARATION

### Human Resistin Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the Human Resistin Standard with 250  $\mu$ L distilled or deionized water into the vial to give a 10 ng/mL concentration of Standard. Invert and mix gently, let sit for 5 minutes then vortex gently.
2. Label six tubes 5, 2.5, 1.25, 0.625, 0.312, and 0.16 ng/mL. Add 100  $\mu$ L Assay Buffer to each of the six tubes. Prepare serial dilutions by adding 100  $\mu$ L of the 10 ng/mL reconstituted standard to the 5 ng/mL tube, mix well and transfer 100  $\mu$ L of the 5 ng/mL reconstituted standard to the 2.5 ng/mL tube, mix well and transfer 100  $\mu$ L of the 2.5 ng/mL Standard to the 1.25 ng/mL tube, mix well and transfer 100  $\mu$ L of the 1.25 ng/mL Standard to the 0.625 ng/mL tube, mix well and transfer 100  $\mu$ L of the 0.625 ng/mL Standard to the 0.312 ng/mL tube, mix well and transfer 100  $\mu$ L of the 0.312 ng/mL Standard to the 0.16 ng/mL tube and mix well.

Standard Concentration ng/mL	Volume of Deionized Water to Add	Volume of Standard to Add
10	250 $\mu$ L	0

Standard Concentration ng/mL	Volume of Assay Buffer to Add	Volume of Standard to Add
5	100 $\mu$ L	100 $\mu$ L of 10 ng/mL
2.5	100 $\mu$ L	100 $\mu$ L of 5 ng/mL
1.25	100 $\mu$ L	100 $\mu$ L of 2.5 ng/mL
0.625	100 $\mu$ L	100 $\mu$ L of 1.25 ng/mL
0.312	100 $\mu$ L	100 $\mu$ L of 0.625 ng/mL
0.16	100 $\mu$ L	100 $\mu$ L of 0.312 ng/mL

### Human Resistin Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Using a pipette, reconstitute each of the Human Resistin Quality Control 1 and Quality Control 2 with 250  $\mu$ L distilled or deionized water into the vials. Invert and mix gently, let sit for 5 minutes then mix well.

**Note: Do not use a Repeater pipette. Change tip for every dilution. Wet tip with Standard before dispensing. Unused portions of reconstituted Standard and Quality Controls should be aliquoted and stored at  $\leq -20^{\circ}\text{C}$  immediately. Avoid multiple freeze/thaw cycles.**



## X. ASSAY PROCEDURE

**Pre-warm all reagents to room temperature prior to setting up the assay.**

1. Dilute the 10X concentrated Wash Buffer 10 fold by mixing the entire content of each bottle of Wash Buffer with 450 mL deionized or distilled water (Dilute both bottles with 900 mL deionized water).
2. Remove the Microtiter Assay Plate from the foil pouch and add 300  $\mu$ L of diluted Wash Buffer to each well. Incubate at room temperature for 5 minutes. Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several 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.**
3. Add 60  $\mu$ L Assay Buffer into all wells.
4. Add in duplicate 20  $\mu$ L of Assay Buffer to blank wells.
5. Add in duplicate 20  $\mu$ L of Human Resistin Standards in order of ascending concentration to the appropriate wells. Add in duplicate 20  $\mu$ L QC1 and 20  $\mu$ L QC2 to the appropriate wells. Add sequentially 20  $\mu$ L of diluted serum or plasma samples in duplicate to the remaining wells.
6. Cover the plate with plate sealer and incubate at room temperature for 1.5 hours on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400 to 500 rpm.
7. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.
8. Wash wells 3 times with diluted Wash Buffer, 300  $\mu$ L per well per wash. Decant and tap firmly after each wash to remove residual buffer.
9. Add 80  $\mu$ L Detection Antibody to all wells. Cover the plate with plate sealer and incubate at room temperature for 1 hour on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400 to 500 rpm.
10. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.
11. Wash wells 3 times with diluted Wash Buffer, 300  $\mu$ L per well per wash. Decant and tap firmly after each wash to remove residual buffer.

## X. ASSAY PROCEDURE (continued)

12. Add 80  $\mu$ L 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.
13. Remove sealer, decant solutions from the plate, and tap plate to remove the residual fluid.
14. Wash wells 3 times with diluted Wash Buffer, 300  $\mu$ L per well per wash. Decant and tap firmly after each wash to remove residual buffer.
15. Add 80  $\mu$ L of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for **approximately** 5 to 20 minutes. Blue color should be formed in wells of Resistin standards with intensity proportional to increasing concentrations of Resistin.

**NOTE:** Please be aware that the blue 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.

16. Remove sealer and add 80  $\mu$ L Stop Solution [**CAUTION: CORROSIVE SOLUTION**] and 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 highest Resistin standard should be approximately 2.5-3.2, or not to exceed the capability of the plate reader used.

### Assay Procedure for Human Resistin ELISA kit (Cat. # EZHR-95K)

	Step 1	Step 2	Step 3-4	Step 5	Step 6-8	Step 9	Step 9-11	Step 12	Step 12-14	Step 15	Step 15	Step 16	Step 16
Well #	<b>Dilute each bottle 10X Wash Buffer with 450mL Deionized Water.</b>	<b>Wash plate 1X with 300 <math>\mu</math>L Wash Buffer. Incubate 5 minutes at Room Temperature. Remove residual buffer by tapping smartly on absorbent towels</b>	Assay Buffer	Standards/ Controls/ Samples	<b>Seal, Agitate, Incubate 1.5 hours at Room Temperature. Wash 3X with 300 <math>\mu</math>L Wash Buffer</b>	Detection Ab	<b>Seal, Agitate, Incubate 1 hour at Room Temperature. Wash 3X with 300 <math>\mu</math>L Wash Buffer</b>	Enzyme Solution	<b>Seal, Agitate, Incubate 30 minutes at Room Temperature. Wash 3X with 300 <math>\mu</math>L Wash Buffer</b>	Substrate	<b>Seal, Agitate, Incubate 5 - 20 minutes at Room Temperature.</b>	Stop Solution	<b>Read Absorbance at 450 nm and 590 nm.</b>
A1, B1			80 $\mu$ L			80 $\mu$ L		80 $\mu$ L		80 $\mu$ L			
C1, D1			60 $\mu$ L	20 $\mu$ L of 0.16 ng/mL Standard									
E1, F1			60 $\mu$ L	20 $\mu$ L of 0.312 ng/mL Standard									
G1, H1			60 $\mu$ L	20 $\mu$ L of 0.625 ng/mL Standard									
A2, B2			60 $\mu$ L	20 $\mu$ L of 1.25 ng/mL Standard									
C2, D2			60 $\mu$ L	20 $\mu$ L of 2.5 ng/mL Standard									
E2, F2			60 $\mu$ L	20 $\mu$ L of 5 ng/mL Standard									
G2, H2			60 $\mu$ L	20 $\mu$ L of 10 ng/mL Standard									
A3, B3			60 $\mu$ L	20 $\mu$ L of QC I									
C3, D3			60 $\mu$ L	20 $\mu$ L of QC II									
E3, F3			60 $\mu$ L	20 $\mu$ L of Diluted Sample									
G3, H3			60 $\mu$ L	20 $\mu$ L of Diluted Sample									
A4, B4 ↓	60 $\mu$ L	20 $\mu$ L of Diluted Sample											

# XI. MICROTITER PLATE ARRANGEMENT

## Human Resistin ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	1.25 ng/mL	QC 1									
B	Blank	1.25 ng/mL	QC 1									
C	0.16 ng/mL	2.5 ng/mL	QC 2									
D	0.16 ng/mL	2.5 ng/mL	QC 2									
E	0.312 ng/mL	5 ng/mL	Sample									
F	0.312 ng/mL	5 ng/mL	Sample									
G	0.625 ng/mL	10 ng/mL	Sample									
H	0.625 ng/mL	10 ng/mL	Sample									

## XII. CALCULATIONS

The dose-response curve of this assay fits best to a sigmoidal 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 5-parameter logistic function.

It is important to make an appropriate mathematical adjustment to accommodate for the dilution factor.

## XIII. ASSAY CHARACTERISTICS

### A. Sensitivity

The lowest level of Human Resistin that can be detected by this assay is 0.02 ng/mL.

### B. Precision

Within Assay Variation

Sample No.	Mean Resistin Levels, ng/mL	Within %CV
1	64.1	4.0
2	12.9	3.2
3	20.2	7.0
4	10.2	3.8

Between Assay Variation

Sample No.	Mean Resistin Levels, ng/mL	Between %CV
1	0.67	7.7
2	3.73	7.1

The assay variations of EMD MILLIPORE Human Resistin ELISA Kits were studied on human serum samples with varying concentrations of resistin. The mean within variation was calculated from results of ten duplicate determinations in each assay of the indicated samples. The mean between variations of each sample was calculated from results of three separate assays with duplicate samples in each assay.

### XIII. ASSAY CHARACTERISTICS (continued)

#### C. Recovery

##### Spike & Recovery of Resistin in Human Serum

Sample No.	Resistin added ng/mL	Expected ng/mL	Observed ng/mL	% of Recovery
1	0	1.17	1.17	100
	0.2	1.37	1.36	99.3
	1.0	2.17	1.96	90.3
	5.0	6.17	5.60	90.8
2	0	0.68	0.68	100
	0.2	0.88	0.91	103
	1.0	1.68	1.54	91.7
	5.0	5.68	5.30	93.3
3	0	1.49	1.49	100
	0.2	1.69	1.68	99.4
	1.0	2.49	2.46	98.8
	5.0	6.49	5.98	92.1
4	0	0.69	0.69	100
	0.2	0.89	0.96	108
	1.0	1.69	1.68	99.4
	5.0	5.69	5.31	93.3

Varying concentrations of human resistin were added to four human serum samples and the resistin content was determined by four separate assays. Percent recovery = observed Resistin concentrations ÷ expected Resistin concentrations x 100%.

### XIII. ASSAY CHARACTERISTICS (continued)

#### D. Linearity

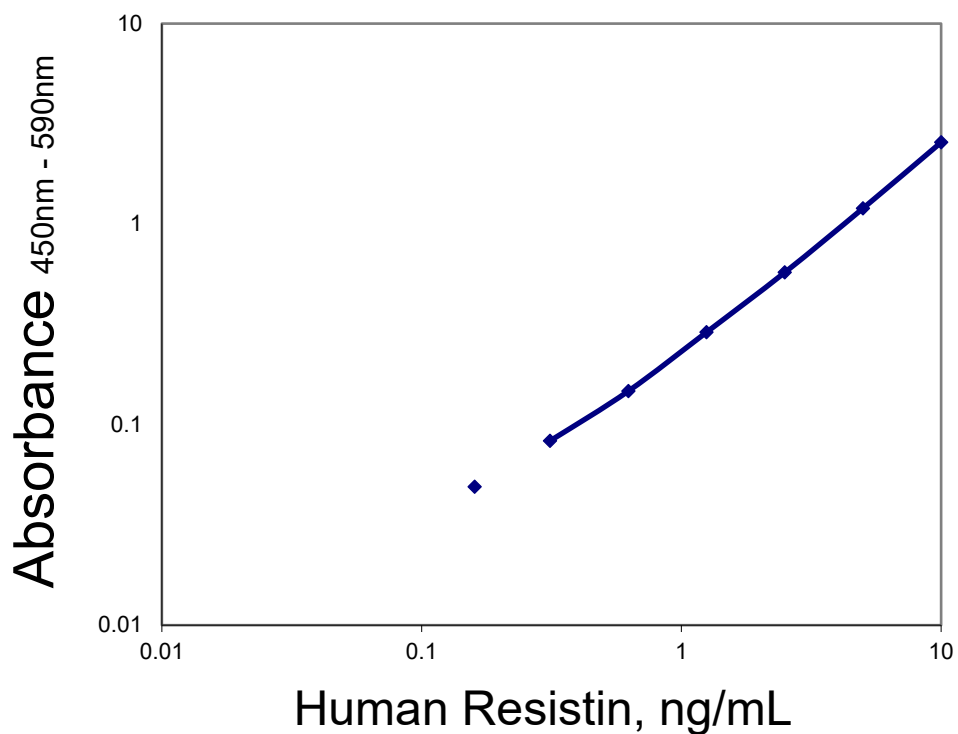
##### Effect of Serum Dilution

Sample No.	Sample Dilution	Expected ng/mL	Observed ng/mL	% Of Expected
1	0	2.53	2.53	100
	2	1.16	1.27	109
	4	0.60	0.64	107
	8	0.32	0.32	100
2	0	1.49	1.49	100
	2	0.75	0.63	84
	4	0.37	0.31	84
	8	0.19	0.17	89
3	0	2.04	2.04	100
	2	1.02	0.86	84
	4	0.51	0.48	94
	8	0.26	0.22	85

Three human serum samples with the indicated sample volumes were assayed in three separate experiments. The samples were diluted for 10-fold following the assay protocol. Further dilutions were made and analyzed. The mean resistin levels and percent of expected from four duplicates determinations are shown. % expected = observed/expected x 100%.

#### XIV. HUMAN RESISTIN STANDARD CURVE

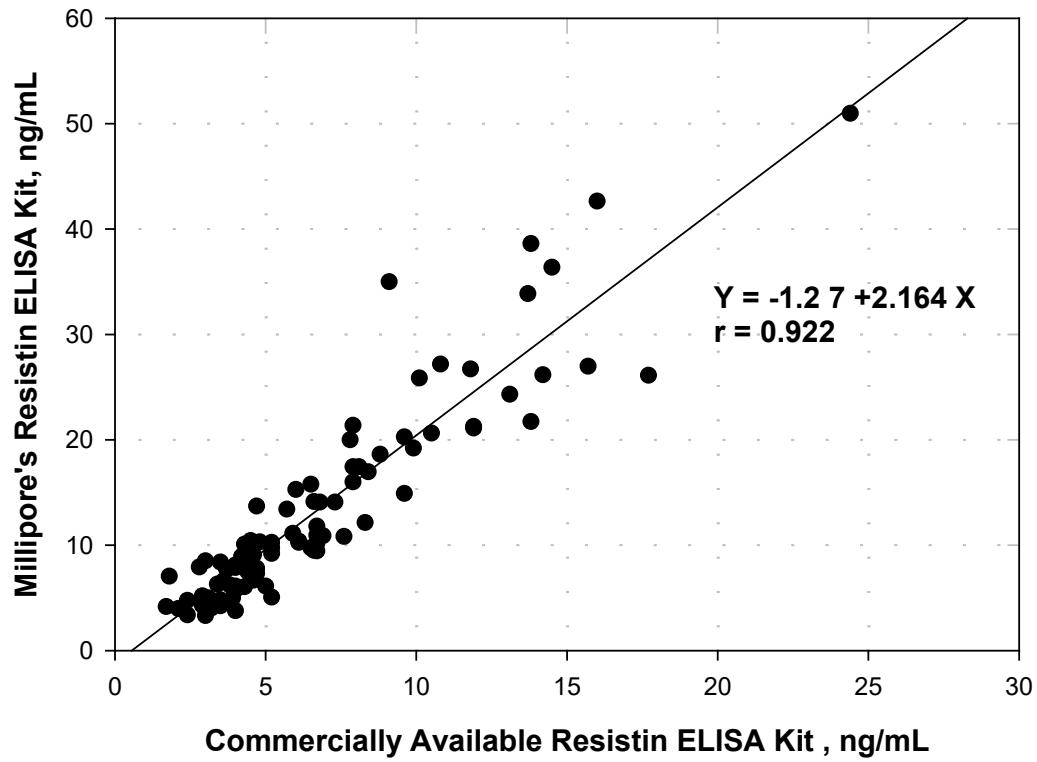
Standard Curve for the Human Resistin ELISA





## XV. METHOD COMPARISON

Millipore's Human Resistin ELISA Kit  
vs. a commercially available  
Human Resistin ELISA Kit



## **XVI. QUALITY CONTROLS**

The ranges for Quality Control 1 and 2 are provided on the card insert or can be located at the EMD Millipore website [emdmillipore.com](http://emdmillipore.com) using the catalog number as the keyword.

## **XVII. TROUBLESHOOTING GUIDE**

1. 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.
2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
3. 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.
4. Avoid cross contamination of any reagents or samples to be used in the assay.
5. Make sure all reagents and samples are added to the bottom of each well.
6. 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.
7. Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. Do not let the absorbency reading of the highest standard reach 3.0 units or higher after acidification.
9. High absorbance in background or blank wells could be due to 1) cross well contamination by standard solution or sample or 2) inadequate washing of wells with Wash Buffer or 3) overexposure to light after substrate has been added.

## XVIII. REFERENCES

1. Tijssen P. "Practice and Theory of Enzyme Immunoassays" in Burdon RH and Knippenberg PH (Ed.), Laboratory Techniques in Biochemistry and Molecular Biology. Amsterdam/NY: Elsevier, 1985
2. Steppan GM et al., 2001. *Nature* 409(18):307.
3. Kim KH et al., 2001. *J. Biol. Chem.* 276(14): 11252.
4. Way JM et al., 2001. *J. Biol. Chem.* 276(28):25651.
5. Smith U and Nagaev I. 2001. *Biochem. Biophys. Res. Comm.* 285 : 561.
6. Moore GB et al., 2001. *Biochem. Biophys. Res. Comm.* 286(4):735.

## XIX. REPLACEMENT REAGENTS

<b>Reagents</b>	<b>Cat. #</b>
Human Resistin ELISA Plate	EP95
10X HRP Wash Buffer Concentrate	EWB-HRP
Human Resistin Standards	E8095-K
Human Resistin Quality Controls 1 and 2	E6095-K
Assay Buffer	EABIR
Human Resistin Detection Antibody	E1095
Enzyme Solution	EHRP-88
Substrate	ESS-TMB5
Stop Solution	ET-TMB
10-pack of Human Resistin ELISA kits	EZHR-95BK

## XX. ORDERING INFORMATION

To place an order or to obtain additional information about our immunoassay products, please contact your Customer Service or Technical Support Specialist.

Contact information for each region can be found on our website:

[emdmillipore.com/contact](http://emdmillipore.com/contact)

### Conditions of Sale

For Research Use Only. Not for Use in Diagnostic Procedures.

### Safety Data Sheets (SDS)

Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at [emdmillipore.com/msds](http://emdmillipore.com/msds).