

# Human Resistin ELISA Kit

Catalog Number KHP0051 (96 tests)

Pub. No. MAN0009568 Rev. 3.0 (30)

**CAUTION!** This kit contains materials with small quantities of Proclin™ 300. Proclin™ 300 is toxic, corrosive, and a skin irritant. Avoid ingestion and contact with eyes, skin and mucous membranes. Observe all federal, state, and local regulations for disposal.

**Note:** For safety and biohazard guidelines, see the “Safety” appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Product description

The Invitrogen™ Human Resistin ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human resistin in serum, plasma, and cell culture supernatant. The assay will recognize both natural and recombinant human resistin.

Resistin is an adipocyte-derived peptide first identified during a search for targets of thiazolidinediones. It has been shown to affect various physiological processes, including insulin response and inflammatory processes within the human body.

## Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KHP0051 (96 tests)
Hu Resistin Standard, lyophilized	1 vial
ELISA Buffer (10X)	2 × 30 mL
Antibody Coated Wells, 96-well plate	1 plate
Detection Antibody	30 µL
HRP labeled Streptavidin, lyophilized	2 µg
Wash Buffer Concentrate (10X)	2 × 30 mL
TMB Substrate Solution	12 mL
Stop Solution	12 mL
Adhesive Plate Covers	2

## Materials required but not supplied

- Distilled or deionized water
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer—automated or manual (squirt bottle, manifold dispenser, or equivalent)

## Before you begin

**IMPORTANT!** Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at [thermofisher.com](http://thermofisher.com).
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

## Prepare 1X Wash Buffer

1. Dilute 30 mL of Wash Buffer Concentrate (10X) with 270 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

## Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at [thermofisher.com](http://thermofisher.com) for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes. Thirty minutes after collection, centrifuge for 15 minutes at 1,000 × g.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

## Pre-dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

- Perform sample dilutions with Standard Diluent Buffer.
- Dilute **serum** and **plasma** samples at least 20-fold in 1X ELISA Buffer.

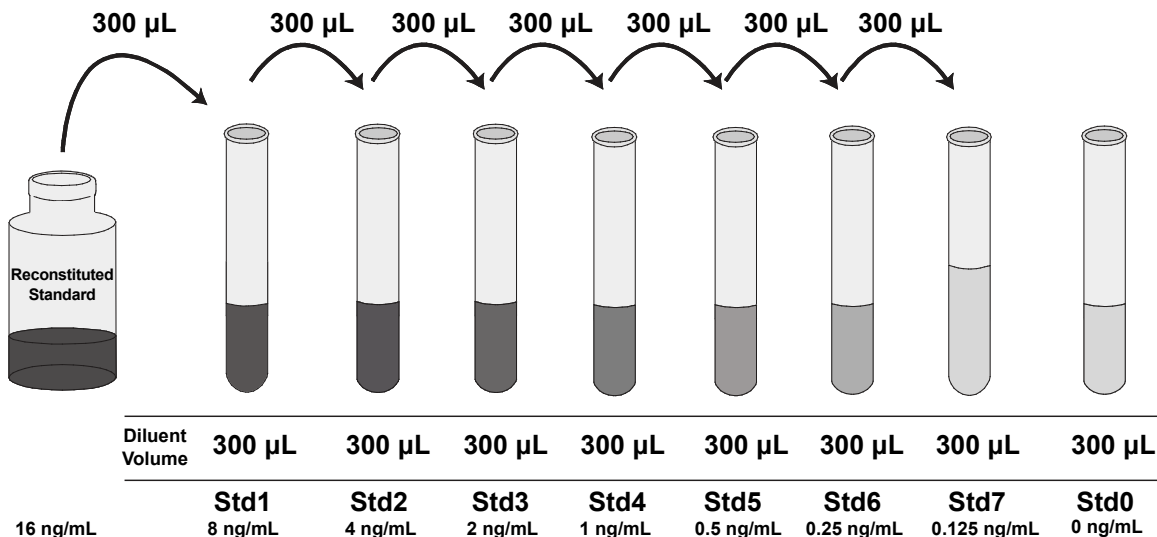
## Prepare 1X ELISA Buffer

Dilute 10X ELISA Buffer 1:10 (e.g., 20 mL 10X ELISA Buffer with 180 mL of deionized water). Label as 1X ELISA Buffer.

## Dilute standards

**Note:** Use glass or plastic tubes for diluting standards.

1. Reconstitute Human Resistin Standard to 16 ng/mL with 1 mL of deionized water. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 15 minutes to ensure complete reconstitution. Label as 16 ng/mL Human Resistin.
2. Add 300  $\mu$ L 1X ELISA Buffer to each of 8 tubes labeled as follows: 8, 4, 2, 1, 0.5, 0.25, 0.125, and 0 ng/mL Human Resistin.
3. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
4. Discard any remaining reconstituted standard.



## Prepare 1X Detection Antibody solution

Dilute Detection Antibody 1:2000 in 1X ELISA Buffer (e.g., 5  $\mu$ L Detection Antibody with 10 mL 1X ELISA Buffer). Label as 1X Detector Antibody.

**Note:** The diluted Detection Antibody is not stable and cannot be stored.

## Prepare 1X HRP labeled Streptavidin solution

**Note:** Prepare within 15 minutes of use. Diluted HRP-labeled Streptavidin is not stable and cannot be stored.

1. Reconstitute HRP Labeled Streptavidin with 100  $\mu$ L 1X ELISA Buffer. After reconstitution, aliquots can be stored at  $-20^{\circ}\text{C}$ . Avoid freeze/thaw cycles.
2. Dilute reconstituted HRP Labeled Streptavidin to the working concentration by adding 25  $\mu$ L in 10 mL (1:400) of 1X ELISA Buffer.

## Perform ELISA (Total assay time: 3.2 hours)

**IMPORTANT!** Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for up to 1 month.



<b>1</b>	<b>Bind antigen</b>	<ol style="list-style-type: none"> <li>Add 100 µL of standards, controls, or samples (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.</li> <li>Cover the plate with a plate cover and incubate 1 hour at 37°C.</li> <li>Thoroughly aspirate the solution and wash wells 3 times with 1X Wash Buffer.</li> </ol>
<b>2</b>	<b>Add detector antibody</b>	<ol style="list-style-type: none"> <li>Add 100 µL of Hu Resistin Detection Antibody solution into each well except the chromogen blanks.</li> <li>Cover the plate with a plate cover and incubate 1 hour at 37°C.</li> <li>Thoroughly aspirate the solution and wash wells 3 times with 1X Wash Buffer.</li> </ol>
<b>3</b>	<b>Add IgG HRP</b>	<ol style="list-style-type: none"> <li>Add 100 µL 1X HRP labeled Streptavidin into each well except the chromogen blanks.</li> <li>Cover the plate with plate cover and incubate for 1 hour at 37°C.</li> <li>Thoroughly aspirate the solution and wash wells 5 times with 1X Wash Buffer.</li> </ol>
<b>4</b>	<b>Add Substrate Solution</b>	<ol style="list-style-type: none"> <li>Add 100 µL Substrate Solution to each well. The substrate solution begins to turn blue.</li> <li>Incubate for 10 minutes at room temperature in the dark.</li> </ol> <p><b>Note:</b> TMB should not touch aluminum foil or other metals.</p>
<b>5</b>	<b>Add Stop Solution</b>	Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

### Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 30 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

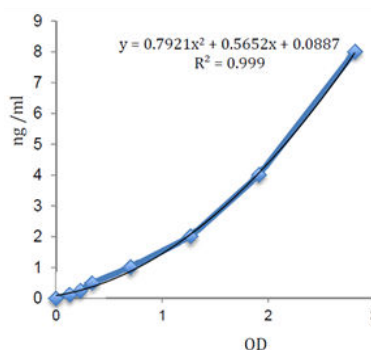
**Note:** Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

### Performance characteristics

#### Standard curve example

Typical standard curve over the range of 0 to 8 ng/mL human resistin.

Standard Human Resistin (ng/mL)	Optical Density (450 nm)
8	2.82
4	1.91
2	1.27
1	0.70
0.5	0.34
0.25	0.23
0.125	0.13
0	0



## Inter-assay precision

Five samples of known human resistin concentration were assayed in replicates of 10 to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Mean (µg/mL)	6.80	22.96	6.49	15.32	25.66
Standard Deviation	0.49	1.24	0.27	1.07	1.11
% Coefficient of Variation	7.20	5.40	4.20	6.97	4.35

## Intra-assay precision

Five samples of known human resistin concentration were assayed in replicates of 10 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Mean (µg/mL)	10.78	19.23	21.49	5.19	12.57
Standard Deviation	0.31	0.99	0.67	0.20	0.47
% Coefficient of Variation	2.86	5.17	3.12	3.77	3.73

## Expected values

human resistin levels in plasma and serum range from 1 to >20 ng/mL (from healthy donors).

## Specificity

This ELISA is specific for the measurement of natural and recombinant human resistin. It does not cross-react with **Human** leptin, RELM-β or adiponectin; **Mouse** resistin, RELM-α or RELM-β; **Rat** resistin or RELM-α.

## Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at [www.thermofisher.com/us/en/home/global/terms-and-conditions.html](http://www.thermofisher.com/us/en/home/global/terms-and-conditions.html). If you have any questions, please contact Life Technologies at [www.thermofisher.com/support](http://www.thermofisher.com/support).

## Recovery

When samples (serum or plasma) are spiked with known concentrations of human resistin, the recovery averages 96% (range 93-108%).

Type	Sample	Average % Recovery	% Range
Serum	1	94.4	93-96
	2	96.6	95-97
	3	98.9	92-108

## Linearity of dilution








Different human serum samples containing human resistin were diluted several fold (1:10 to 1:40).

Sample	Sample Dilution	Expected (ng/mL)	Observed (ng/mL)	% of Expected
1	1:10	5.59	5.59	100
	1:20	2.80	2.63	94.15
	1:40	1.40	1.23	88.29
2	1:10	6.13	6.13	100
	1:20	3.06	3.71	121.11
	1:40	1.53	1.80	117.13
3	1:10	6.33	6.33	100
	1:20	3.16	3.66	115.66
	1:40	1.58	1.63	102.90

## Sensitivity

The analytical sensitivity of this assay is 100 pg/mL human resistin.

### Product label explanation of symbols and warnings

 REF	Catalog Number	 LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents
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**Manufacturer's address:** Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

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