## PROTOCOL OVERVIEW

DRG Human Resistin ELISA Kit (EIA-4945) is designed to measure the concentration of human Resistin from human serum/plasma, human adipocytes, or conditioned medium.

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

The immunoplate in this kit is pre-coated with Anti-Human Resistin Capture Antibody and the nonspecific binding sites are blocked. The Human Resistin in the sample or in the standard solution can bind to the capture antibody immobilized in the wells. After washing procedure, the biotinylated anti-Human Resistin Detection Antibody which can bind to the Human Resistin trapped in the wells is added. After washing, the Streptavidin-Horseradish Peroxidase (SA-HRP) which catalyzes the substrate solution (TMB) is added. The enzyme-substrate reaction is terminated by the addition of a stop solution. The intensity of the color is directly proportional to the amount of Human Resistin in the standard solutions or samples. A standard curve of Human Resistin with known concentration can be established accordingly. The Human Resistin with unknown concentration in samples can be determined by extrapolation to this standard curve.

## ASSAY CONDITIONS:

Plasma, serum, culture media, tissue homogenate, CSF, urine or any biological fluid can be assayed as long as the level of the sample is high enough for the sensitivity of the kit to detect it.

## STORAGE

1. Store the kit at 4°C upon receipt. The kit will be stable for 6 months. The kit should be equilibrated to room temperature (20-23°C) before assay.
2. Store **1x** assay buffer at 4°C.
3. Remove any unneeded strips from Human Resistin Antibody-Coated plate, reseal them in zip-lock foil and keep at 4°C.
4. Keep rehydrated solution of Human Resistin Standard, Biotinylated anti-Human Resistin Detection Antibody and HRP at 4°C. Prepare only the required amount.

## NOTE:

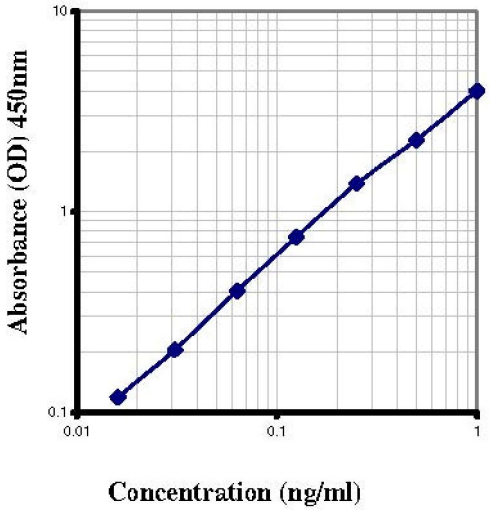
1. It is recommended that the solutions be used on the same day of rehydration.
2. Un-extracted plasma sample of normal subjects diluted with **1x** assay buffer at 1:20 for assay is recommended
3. After adding Stop buffer, read the plate within 20 minutes.

## CALCULATION OF RESULTS

Plot the standard curve on log-log graph paper. Known concentrations of Human Resistin Standard and its corresponding O.D. reading is plotted on the log scale (X-axis) and the log scale (Y-axis) respectively. The standard curve shows a correlated relationship between Human Resistin concentrations and the corresponding O.D. absorbance. As the standard concentration increases, the intensity of the blue color, and in turn the O.D. absorbance, increases.

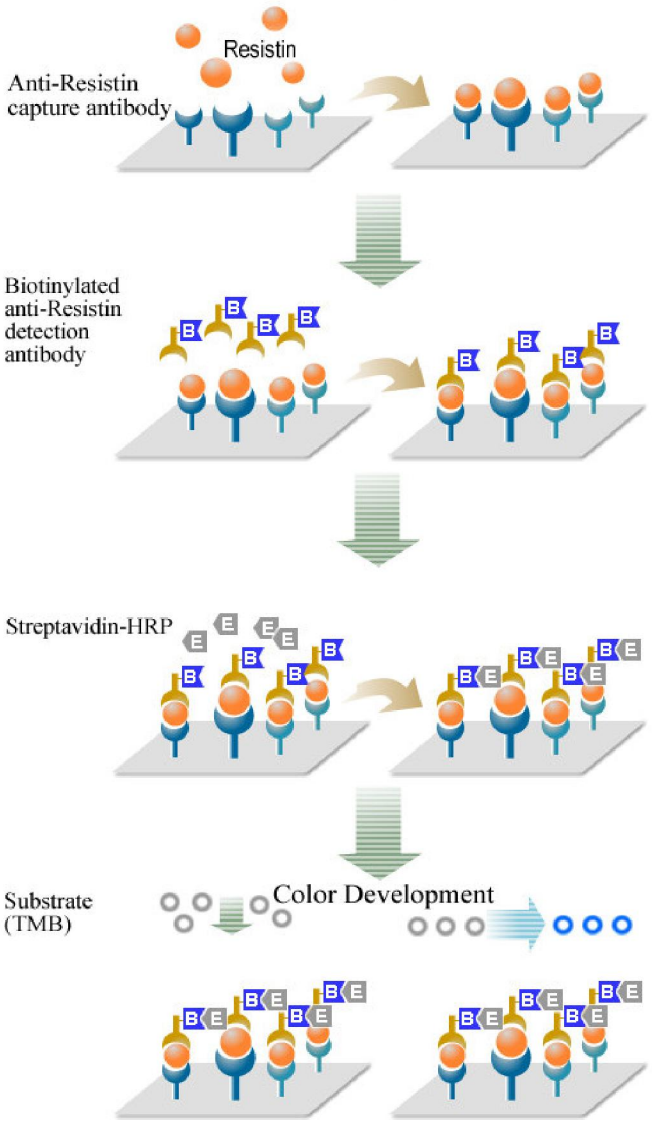
The concentration of Human Resistin in a sample is determined by plotting the sample's O.D. on the Y-axis, then drawing a horizontal line to intersect with the standard curve. A vertical line dropped from this point will intersect the X-axis at a coordinate corresponding to the Human Resistin concentration in the unknown sample.
Refer to QC Data sheet for acceptable values of the positive control.

**Human Resistin Standard Curve**




**CAUTION**: DRG guarantees that its products conform to the information contained in this publication. The purchaser must determine the suitability of the product for its particular use**.**

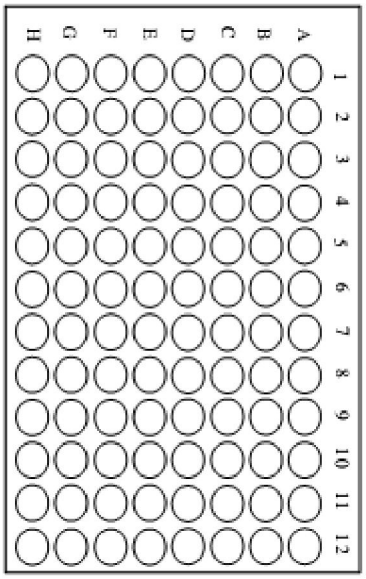
Assay Principle



## LIST OF COMPONENTS

**(ATTENTION: Store all components at 4°C. DO NOT FREEZE.)**

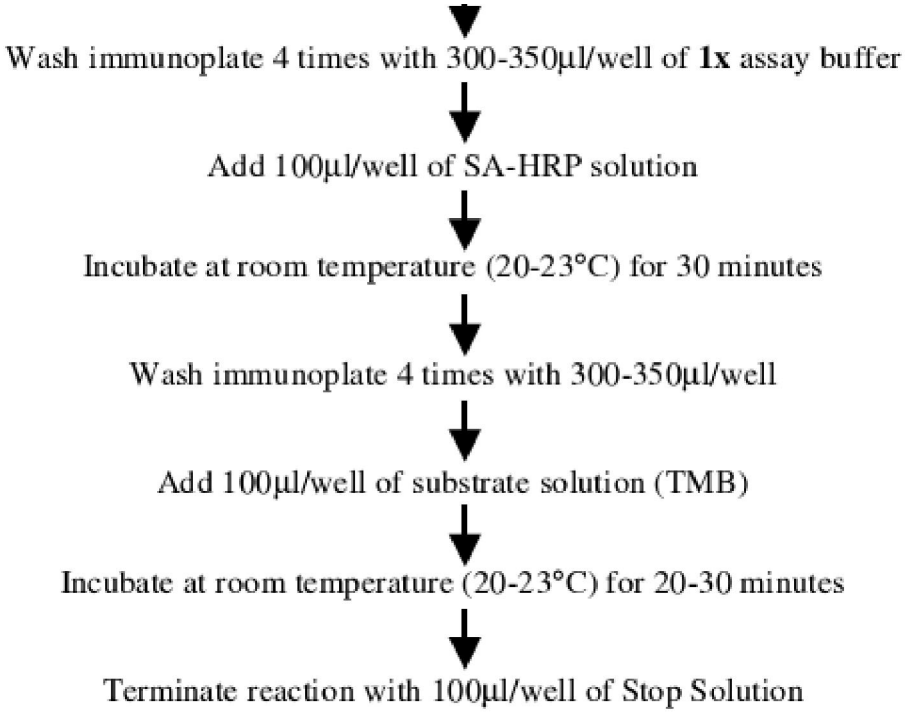
1. 20x Assay Buffer concentrate *(50ml)*
2. 96 Well Anti-Human Resistin - Capture Antibody-Coated Plate *(1 Plate)*
3. Human Resistin Standard *(10ng/vial)*
4. Biotinylated Anti-Human Resistin - Detection Antibody *(1vial)*
5. Human Resistin Positive Control *(2vials)*
6. Streptadivin-horseradish peroxidase - (SA-HRP)*(30µl)*
7. Substrate Solution (TMB) *(12ml)*
8. Stop Solution 2N HCL *(15ml)*
9. Acetate plate sealer (APS) *(3 pieces)*
10. Assay Diagram *(1 sheet)*:



## MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettor (s) and disposable pipette tips
2. Multi-channel pipette capable of dispensing 50-100µl
3. Solution reservoir *(recommended)*
4. Microtiter plate washer *(recommended)*
5. Orbital plate shaker capable of 300-500 rpm *(recommended)*
6. Microtiter plate reader capable of absorbance measurement between 450 nm-650 nm
7. Well-closed containers (15 ml tubes or more in capacity)
8. Absorbent material for blotting.

## SUMMARY OF ASSAY PROTOCOL:




## ADDITIONAL RECOMMENDED PROCEDURAL NOTES:

1. Reagents of different lot numbers should not be mixed.
2. Recheck the reagent labels when loading the plate to ensure that everything is added correctly.
3. Unused microplate strips should be placed back in the foil pouch with a desiccant and stored at 4ºC. Do not allow moisture to enter the wells.
4. When handling the plate, avoid touching the bottom.
5. Manual washing may cause high duplicate coefficient variations. To reduce this factor, liquid from the plate should be removed by inverting and blotting the plate on an absorbent material.
6. If the room temperature is not within the suggested range (20 - 23°C), variations in results may

occur.

7. The same reservoir for the reagents may be reused if the reservoir is washed well with distilled water before each use.
8. *Each laboratory must determine the appropriate dilution factors for the samples to be measured to ensure that the samples are within the dynamic range of the standard curve.*
9. High levels of interfering proteins may cause variations within the sample results; therefore, it is imperative to select the appropriate sample preparation procedure to obtain the optimal results.
10. Each time a new tip is used, make sure the tip is secure and free of air bubbles. For better intra-assay variation, aspirate and expel a reagent or sample back into the container a few times prior to loading.
11. Avoid submerging the whole tip into reagents because droplets can accumulate at the end of the tip causing an excess of reagent to be loaded into the well. This can lead to poor results.
12. A multi-channel pipette is **NOT** recommended to load the biotinylated detection antibody or standard because variations in results may occur.
13. For optimal results, an orbital plate shaker capable of 300-500 rpm. is recommended for all incubations.
14. Modification of the existing protocol (*i.e.* standard dilutions, pi-petting technique, washing technique, incubation time or temperature, storage conditions, and kit expiration) may affect the sensitivity and specificity of the test.

## REAGENT PREPARATION

**Note:** *The kit should be equilibrated to room temperature (20-23ºC) before opening any vials and starting the assay. It is highly recommended that the solutions be used as soon as possible after rehydration.*

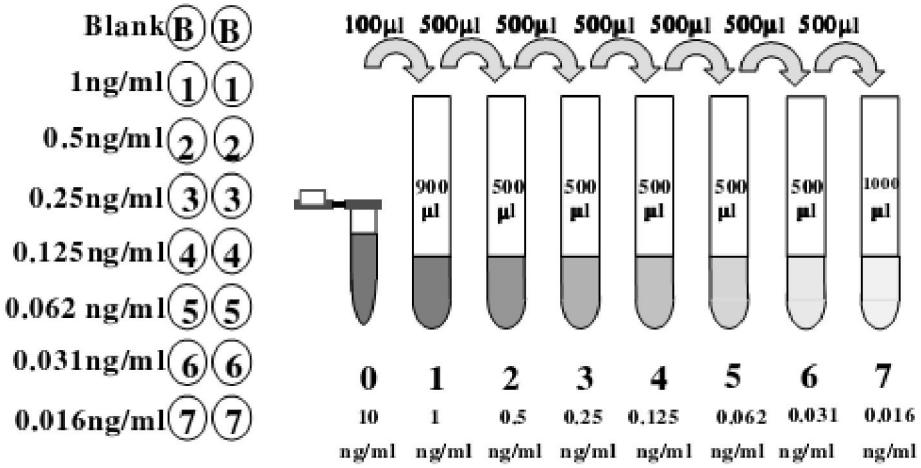
1. **1x Assay buffer:** Dilute the **20x** assay buffer concentrate with 950ml of distilled water. This assay buffer will be used to wash the plate and reconstitute all of the other compounds in this kit. If crystals are observed in the **20x** Assay buffer, warm the bottle in a 37°C water bath for approximately 30 minutes or until the crystals disappear. After preparation, store **1x** Assay buffer at 4°C.
2. **Biotinylated anti-Human Resistin Detection Antibody:** Rehydrate biotinylated anti-Human Resistin Detection Antibody with 100µl of **1x** assay buffer, vortex (centrifuge the tube to dislodge powder from the cap or walls). Dilute biotinylated anti-Human Resistin Detection Antibody to 1:200 and mix thoroughly before use.
3. **Streptavidin-Horseradish Peroxidase (SA-HRP):** Centrifuge the HRP vial (30µl) provided in this kit (3,0005,000 rpm, 5 seconds) and dilute HRP with **1x** assay buffer to 1:2000 before use. Vortex thoroughly.
4. **Human Resistin Positive Control:** Rehydrate Human Resistin Positive Control with 300µl of **1x** assay buffer (centrifuge the tube to dislodge powder from cap or walls). Vortex thoroughly.

## HUMAN RESISTIN STANDARD PREPARATION

1. Rehydrate recombinant Human Resistin standard with 1 ml **1x** assay buffer, vortex. Allow the solution to sit at least 10 minutes at room temperature (20-23°C) to completely dissolve in solution. Vortex and centrifuge before use. The concentration of this stock solution is 10 ng/ml.

2. Prepare Human Resistin standard solutions as follows:

| Standard No. | Std. volume Assay Buffer | | Concentrations |
|---|---|---|---|
| Stock | Powder | 1000µl | 10ng/ml |
| #1 | 100µl of Stock | 900µl | 1ng/ml |
| #2 | 500µl of #1 | 500µl | 0.5ng/ml |
| #3 | 500µl of #2 | 500µl | 0.25ng/ml |
| #4 | 500µl of #3 | 500µl | 0.125ng/ml |
| #5 | 500µl of #4 | 500µl | 0.062ng/ml |
| #6 | 500µl of #5 | 500µl | 0.031ng/ml |
| #7 | 500µl of #6 | 500µl | 0.016ng/ml |




## HUMAN RESISTIN ELISA PROTOCOL

1. Thoroughly read this protocol before performing an assay. Allow all reagents to come to room temperature (20-23°C) prior to the start of the assay.
2. Remove Capture Antibody-Coated Plate from its zip-lock foil pouch. Remove any unneeded strips from the plate frame, reseal them in the foil pouch, and return the foil pouch to 4°C.
3. Add 100µl of the prepared Human Resistin Standard solutions from #7 to #1 (reverse order of serial dilution) in duplicate to each well.

4. Leave wells A-1 and A-2 empty as Blank.
5. Add 100µl of Human Resistin Positive control solution in duplicate.
6. Add 100µl diluted samples into their designated wells.
7. Seal the immunoplate with acetate plate sealer (APS). Incubate for 2 hours at room temperature (20-23ºC) on a plate shaker (300400 rpm).
8. Before washing the plate, remove the plate sealer carefully. Completely discard the liquid from wells. Wash each well with 300350µl assay buffer four times. At the end of the wash, discard the buffer, invert the plate and tap on a clean absorbent towel.
9. Add 100µl biotinylated anti-Human Resistin Detection Antibody into each well **except the Blank**. Reseal the immunoplate with plate sealer and incubate for 2 hours at room temperature (2023ºC) on a plate shaker (300-400 rpm).
10. Wash 4 times with the **1x** assay buffer as described in step 8.
11. Add 100µl SA-HRP solution into each well. Reseal the immunoplate with plate sealer and incubate the plate for 30 minutes at room temperature (20-23ºC) on a plate shaker (300-400 rpm).
12. Wash 4 times with the **1x** assay buffer as descried in step 8.
13. Add 100µl substrate solution (TMB) provided in this kit into each well. Reseal the plate with plate sealer to protect from light and incubate the plate for 20-30 minutes at room temperature (20-23ºC) on a plate shaker (300-400 rpm).
14. Add 100µl Stop Solution (2N Hydrochloric Acid) into each well to stop the reaction. The color in the well should change from blue to yellow. If the color change does not appear to be uni form, gently tap the plate to ensure thorough mixing. Go to the next step within 20 minutes.
15. Read absorbance O.D. at 450 nm using a Microtiter Plate Reader.

**REFERENCES:**

1. Steppan, C.M. et al. 2001. The hormone resistin links obesity to diabetes. Nature. 409:307-312.
2. Steppan, C.M. et al. 2001. A family of tissue-specific resistin-like molecules. Proc. Natl. Acad. Sci. U. S. A. 98:502-506.
3. Way, J.M. et al. 2001. Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor gamma agonists. J. Biol. Chem. 276:25651-25653.