



**Assay Solution**  
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# Human AgRP ELISA Kit

**Catalog #: AYQ-E10311 (96 wells)**

User Manual

*This kit is designed to quantitatively detect the levels of Human AgRP in cell lysates, serum/ plasma and other suitable sample solution.*

**Manufactured and Distributed by:**

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**FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES**

## Important notes

Before using this product, please read this manual carefully; after reading the subsequent contents of this manual, please note the following specially:

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommend that all standards, testing samples are tested in duplicate.
- Using serial diluted sample is recommended for first test to get the best dilution factor.
- If the blue color develops too light after 15 minutes incubation with the substrate, it may be appropriate to extend the incubation time (Do not over-develop).
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate Solution.
- TMB developing agent is light-sensitive. Avoid prolonged exposure to the light.

**ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS.**

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|--------------------|--|
| <b>Catalog#</b>    | AYQ-E10311   |
| <b>Name</b>        | Human AgRP ELISA kit   |
| <b>Size</b>        | 96 T   |
| <b>Storage</b>     | Store the unopened product at 2 - 8° C. Protect from light. Do not use past expiration date.   |
| <b>Gene ID</b>     | 181  |
| <b>Gene Symbol</b> | AgRP   |
| <b>Synonym</b>     | agouti related protein homolog (mouse); agouti-related protein; AgRP; Agrt; AGRTMGC118963; ART; ARTagouti (mouse) related protein; ASIP2 |
| <b>Species</b>     | Human  |

## Scientific Background

Agouti-related protein (AgRP), also called agouti-related peptide, is a neuropeptide produced in the brain by the AgRP/NPY neuron. It is synthesized only in neuropeptide Y (NPY)-containing cell bodies located in the ventromedial part of the arcuate nucleus in the hypothalamus. AgRP is co-expressed with NPY and acts to increase appetite and decrease metabolism and energy expenditure. It is one of the most potent and long-lasting of appetite stimulators. In humans, the agouti-related peptide is encoded by the AGRP gene.

Agouti-related protein is expressed primarily in the adrenal gland, subthalamic nucleus, and hypothalamus, with lower levels of expression in the testis, kidneys, and lungs. The appetite-stimulating effects of AgRP are inhibited by the hormone leptin and activated by the hormone ghrelin. Adipocytes secrete leptin in response to food intake. This hormone acts in the arcuate nucleus and inhibits the AgRP/NPY neuron from releasing orexigenic peptides. Ghrelin has receptors on NPY/AgRP neurons that stimulate the secretion of NPY and AgRP to increase appetite. AgRP is stored in intracellular secretory granules and is secreted via a regulated pathway. The transcriptional and secretory action of AgRP is regulated by inflammatory signals. Levels of AgRP are increased during periods of fasting. It has been found that AgRP stimulates the hypothalamic-pituitary-adrenocortical axis to release ACTH, cortisol and prolactin. It also enhances the ACTH response to IL-1-beta, suggesting it may play a role in the modulation of neuroendocrine response to inflammation. Conversely, AgRP-secreting neurons inhibit the release of TRH from the PVN, which may contribute to conservation of energy in starvation. This pathway is part of a feedback loop, since TRH-secreting neurons from PVN stimulate AgRP neurons.

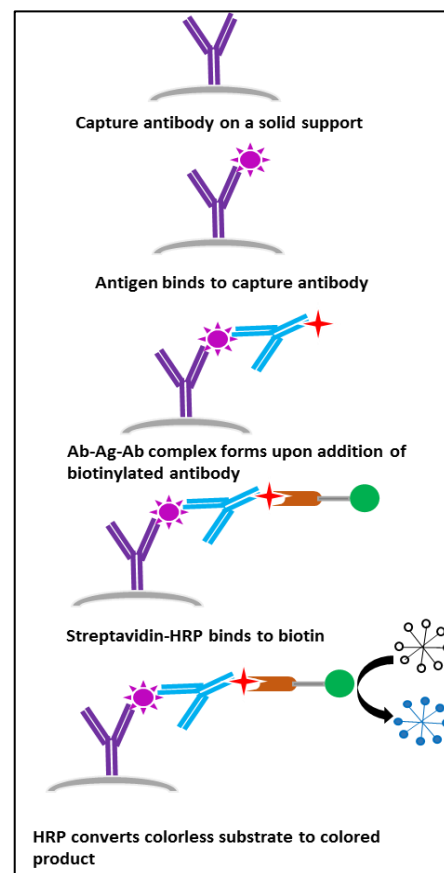
## Intended use

The kit is used to quantify the Human AgRP in serum/ plasma, cell culture supernatant and other suitable sample solution.

|                       |                   |
|-----------------------|-------------------|
| <b>Standard range</b> | 62.5 – 4000 pg/mL |
| <b>Assay time</b>     | 4.5 hours         |
| <b>Validity</b>       | Six months        |
| <b>Store at</b>       | 2-8 °C            |

## Assay principle

The Human AgRP ELISA Kit is based on standard sandwich enzyme-linked immunosorbent assay technology. Anti-Human AgRP specific antibody has been pre-coated onto 96-well plate. Human AgRP present in the standards/ samples bind to the capture antibody. Subsequently, biotinylated anti-Human AgRP detection antibody is added to form an Ab-Ag-Ab sandwich. After a washing step, streptavidin-HRP is added and the unbound conjugate is removed with wash buffer. Next, addition of HRP substrate, TMB, results in the production of a blue colored product that changes to yellow after the addition of acidic Stop Solution. The density of yellow color is directly proportional to the amount of Human AgRP captured on plate.



## Materials supplied

|  |                       |
|--|-----------------------|
| 1. Human AgRP standard:                              | 4 ng/vial ×2          |
| 2. 96-well plate pre-coated with anti-Human AgRP Ab: | 1                     |
| 3. Sample Diluent buffer :                           | 12 ml × 2             |
| 4. Detection antibody:                               | 1 vial, dilution 1:60 |
| 5. Streptavidin-HRP:                                 | 1 vial dilution 1:40  |
| 6. Antibody Diluent buffer:                          | 12 ml                 |
| 7. Streptavidin HRP diluent buffer:                  | 12 ml                 |
| 8. Chromogen Solution A:                             | 6 ml                  |
| 9. Chromogen Solution B:                             | 6 ml                  |
| 10. Stop Solution:                                   | 6 ml                  |
| 11. 20 × Wash Buffer:                                | 25 ml                 |
| 12. Plate sealers                                    | 2                     |
| 13. Package insert                                   | 1                     |

## Materials required but not supplied

- 1x PBS.
- Standard plate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Distilled water.
- Absorbent paper.
- Materials used for sample preparation.

## Sample Preparation and storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- Cell culture supernatant, tissue lysate or body fluids: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C
- Serum: Allow the serum to clot in a serum separator tube (about 4hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.
- Plasma: Collect plasma using heparin as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 minutes of collection. Analyze immediately or aliquot and store frozen at -20°C. EDTA and citrate are not recommended as the anticoagulant.

## Reagent Preparation

### Standard

- Human AgRP: Standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard (4 ng /vial) are included in each kit. Use one tube for each experiment.
- Prepare 4000 pg/ml → 62.5 pg/ml of Human AgRP standard solutions:
- Add 1.0 ml of Sample Diluent Buffer into one standard vial with 4 ng Human AgRP. Keep the tube at room temperature for 10 minutes and mix thoroughly. This is 4000 pg/ml standard solution.
- Label 6 Eppendorf tubes with 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml respectively. Then make 2-fold serial dilution from 4000 pg/ml to 62.5 pg/ml in 1.5 ml tubes with sample diluent buffer.
- Make sure each tube has ≥ 250 µl of standard.

**Note:** *The standard solutions are best used within 2 hours.*

### Preparation of detection anti-Human AgRP antibody working solution

- The stock solution is stable at 2-8 °C for up to 1 month. After opening the vial use within 1 month. For long-term storage, please aliquot and store at -20 °C. Avoid freeze-thaw cycles.
- The working solution should be prepared no more than 2 hours prior to the experiment

- The reagent is supplied as 60X concentrate. Empty the total contents into 10.62 ml of Antibody Diluent Buffer or prepare the solution separately in a volume as needed. The solution should be mixed thoroughly.
- The total volume should be: 0.1 ml/well x the number of wells (Allowing 0.1-0.2 ml more than total volume).

#### **Preparation of Streptavidin-HRP working solution**

- The solution should be prepared no more than 1 hour prior to the experiment.
- The total volume should be: 0.1 ml/well x the number of wells (allowing 0.1-0.2 ml more than total volume).
- Streptavidin-HRP should be diluted 1:40 with Streptavidin-HRP Diluent buffer and mixed thoroughly.

#### **Preparation of TMB Substrate solution**

- The solution should be prepared no more than 10 min prior to the experiment.
- The total volume should be: 0.1 ml/well x the number of wells (allowing 0.1-0.2 ml more than total volume).
- Mix equal volumes of chromogen solutions A and B. Protect from light.

#### **Wash Buffer**

- If crystals have formed in the 20X wash buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
- Dilute 25 ml Wash Buffer Concentrate (20X) to a total volume of 500 ml with distilled water.

### **Assay Procedure**

Bring all reagents to room temperature before use. Human AgRP standard curve should be prepared for each experiment. The user will decide sample dilution factor by rough estimation of Human AgRP concentration in samples.

1. Add 100 µl of sample or standards per well. Add 0.1 ml of the sample diluent into the control well (Zero well). Cover with an adhesive strip and incubate at room temperature for 2 hours.  
Note: We recommend that each Human AgRP standard solution and each sample is measured in duplicate.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 µl of the Detection Antibody working solution to each well. Cover with a new adhesive strip and incubate at room temperature for 2 hours.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 µl of the working solution of Streptavidin-HRP to each well. Cover the plate and incubate at room temperature for 30 min. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2 for three times.

7. Add 100  $\mu$ l of TMB substrate solution to each well. Cover and incubate at room temperature for 5 -15 min until a gradient develops and you see visible color in the 2<sup>nd</sup> lowest concentration well. Protect from light. Do not over-develop.
8. Add 50  $\mu$ l Stop Solution to each well. Mix well.
9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

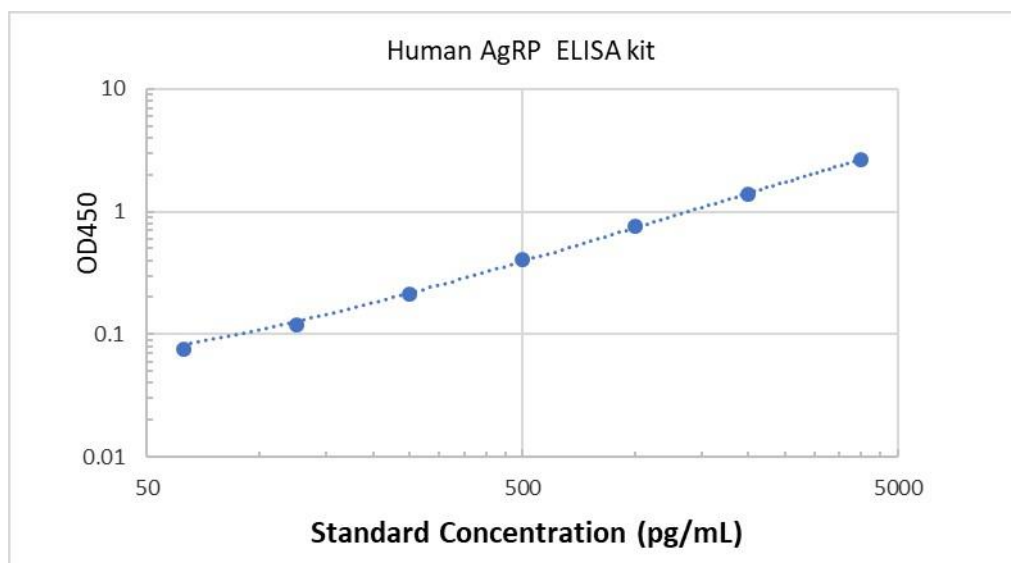
## Result calculation

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human AgRP concentration of the samples can be interpolated from the standard curve.

**Note:** if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution

## Typical data

This standard curve was generated at the AssaySolution laboratory for demonstration purpose only. A standard curve must be run with each assay.



## Sensitivity

The sensitivity or minimum detectable dose (MDD) of Human AgRP was determined to be 50 pg/ml. MDD is defined as the Human AgRP concentration resulting in an O.D.<sub>450</sub> value that is 2 standard deviations higher than blank.



## Spiking and Recovery

Recovery was determined by spiking the following matrices with various concentrations of Human AgRP.

| Sample Type  | Average Recovery (%) | Range (%) |
|--------------|----------------------|-----------|
| Cell lysates | 94.4                 | 89-100    |
| Serum        | 102.7                | 95-109    |

## Reproducibility

- Inter-assay- <10.4%
- Intra-assay- <7.3%

## Specificity

This kit recognizes both natural and recombinant Human AgRP.

## Sample Dilution

The user may need to determine the dilution factor in a preliminary experiment. If required, samples should be diluted in sample diluent buffer.

*For additional troubleshooting information please email us at [info@assaysolution.com](mailto:info@assaysolution.com)*

**Plate Layout**

|           |          |          |          |          |          |          |          |  |
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| <b>4</b>  |          |          |          |          |          |          |          |  |
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| <b>6</b>  |          |          |          |          |          |          |          |  |
| <b>7</b>  |          |          |          |          |          |          |          |  |
| <b>8</b>  |          |          |          |          |          |          |          |  |
| <b>9</b>  |          |          |          |          |          |          |          |  |
| <b>10</b> |          |          |          |          |          |          |          |  |
| <b>11</b> |          |          |          |          |          |          |          |  |
| <b>12</b> |          |          |          |          |          |          |          |  |
| <b>A</b>  | <b>B</b> | <b>C</b> | <b>D</b> | <b>E</b> | <b>F</b> | <b>G</b> | <b>H</b> |  |

# Troubleshooting Information

## High Background

| Probable Cause:                                   | Solution/ Action   |
|---|--|
| <b>High incubation temperature:</b>               | Incubate at room temperature (25 °C) throughout the procedure  |
| <b>Insufficient washing of the plate:</b>         | Fill the wells with wash buffer and aspirate completely for the next wash<br>Increase the number of washes<br>Add soak time (20-30 seconds) in between the washes<br>Use automated plate washer, if available and check that all the channels are operating properly |
| <b>Concentrated streptavidin-HRP</b>              | Streptavidin-HRP was not diluted properly<br>Dilute the streptavidin-HRP as mentioned in the manual  |
| <b>Light exposure during substrate incubation</b> | The TMB substrate is light sensitive and turns to blue color in the presence of light. The incubation must be carried out in dark.   |
| <b>Stop solution not added</b>                    | Color will continue to develop if stop solution is not added   |
| <b>Diluents came with the kit were not used</b>   | Standards/ sample, detection antibody and streptavidin-HRP must be diluted in the respective buffers came with the kit. Do not use buffers from other kits   |
| <b>Contaminated solutions</b>                     | Prepare fresh working solutions  |

## Poor Standard Curve

| Probable Cause:                          | Solution/ Action  |
|--|---|
| <b>Improper standard reconstitution:</b> | Spin the vial briefly before opening<br>Reconstitute the standard as mentioned in the manual. After reconstitution, leave it atleast for 10 minutes at room temperature<br>Do not store and reuse diluted standards |
| <b>Curve fitting problem:</b>            | Log transform the values on both axes<br>Use 4-PL/ 5-PL curve fitting programs  |
| <b>Incubation temperature/ time</b>      | Use the recommended standard incubation conditions  |
| <b>Poor dilutions</b>                    | Pipetting error. Check pipetting technique and calculations.<br>Use calibrated pipettes.  |

## No Signal

| Probable Cause:                           | Solution/ Action   |
|---|--|
| Omission of reagent(s):                   | Read the manual entirely. Check that all the reagents are added in the correct order as stated in the manual |
| Incorrect detection antibody was used:    | Use the detection antibody came with the kit   |
| Chromogen solutions were mixed improperly | Use the recommended procedure to prepare the TMB substrate   |
| HRP inhibitor in sample/ buffers          | Check that the samples/ buffers do not have sodium azide as it will inhibit peroxidase reaction.             |
| Vigorous washing                          | If the washing is done manually, pipette the wash buffer gently.   |
| Dried wells                               | Do not allow the wells to dry out during the assay. Seal with the supplied adhesive cover during incubations |
| Improper plate reader settings            | Check the wavelength and read the plate again  |

## Erratic duplicate OD values

| Probable Cause:                     | Solution/ Action   |
|-------------------------------------|--|
| Insufficient washing of the plate   | Fill the wells with wash buffer and aspirate completely for the next wash<br>Increase number of washes<br>Add soak time (20-30 seconds) in between the washes<br>Use automated plate washer, is available and check that all the channels are functioning properly |
| Poor dilutions                      | Pipetting error. Check pipetting technique and calculations.<br>Use calibrated pipettes.   |
| Improper mixing of samples/ buffers | Mix the samples well before pipetting<br>Thoroughly mix the working solutions of detection antibody/ streptavidin-HRP  |
| Contamination from other wells      | Do not reuse the adhesive covers from previous assay setups<br>Change pipette tips during reagent addition. If same pipette tip is being used to dispense reagents, care should be taken, not to touch the solution in the well                                    |
| Precipitates in the samples/ buffer | If precipitates are visible in wash buffer concentrate, keep it at 37 °C for 10-15 minutes until no precipitates are visible<br>Centrifuge the samples to remove particulate matter  |
| Dried wells                         | Do not allow the wells to dry out during the assay. Seal with the supplied adhesive cover during incubations   |