EN2 ELISA Kit (Human) (OKGD00121)

Instructions For Use

For the quantitative measurement of EN2 in biospecimen.

Lot to lot variation can occur. Refer to the manual provided with the kit.

This product is intended for research use only.

Table of Contents

1. Background

Principle

Aviva Systems Biology EN2 ELISA Kit (Human) (OKGD00121) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific for EN2 has been pre-coated onto a 96-well plate. Standards or test samples are added to the wells. The Biotin conjugated EN2 antibody binds to the captured EN2 on 96 well plate. Next it is incubated with horseradish peroxidase (HRP)-Streptavidin. After washes, an enzymatic reaction is produced through the addition of TMB substrate which is catalyzed by HRP generating a blue color product that changes yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm is proportional to the amount EN2 in the sample.

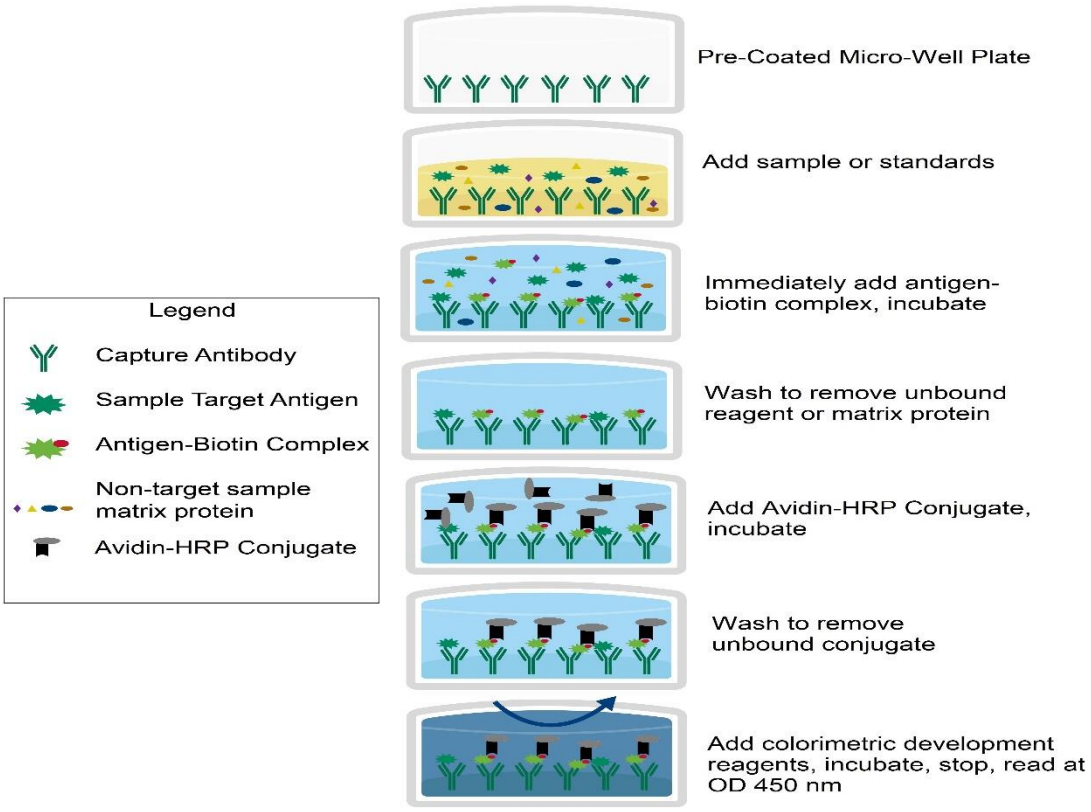
Target Background

Homeobox-containing genes are thought to have a role in controlling development. In Drosophila, the 'engrailed' (en) gene plays an important role during development in segmentation, where it is required for the formation of posterior compartments. Different mutations in the mouse homologs, En1 and En2, produced different developmental defects that frequently are lethal. The human engrailed homologs 1 and 2 encode homeodomain-containing proteins and have been implicated in the control of pattern formation during development of the central nervous system.

General Specifications

General Specifications	
Range	0.47 - 30 ng/ml
Limit of Detection (LOD)	<0.4 ng/ml
Target Information	EN2 (Human) UniProt ID: P19622 Gene ID: 2020 Target Alias: Engrailed Homeobox 2, Homeobox Protein EN-2, Engrailed Homolog 2, Engrailed-2

2. Assay Summary



3. Storage and Stability

Upon receipt, store Biotin-Detection Antibody and HRP-Streptavidin in -20°C kits, rest of Kits at 4°C until expiration date.

4. Kit Components

The following reagents are the provided contents of the kit:

Description	Quantity	Storage Conditions
Human EN2 Biotin Detection Antibody	1 x 30 µL	Store at -20°C
Anti-EN2 Microplate	1 x 96 wells plate	Store at 4°C
EN2 Lyophilized Standard	2 x vials (30 ng/ml/vial)	
Assay Diluent A	1 x 50 mL	
HRP-Streptavidin	1 x 12 µL	
20X Wash Buffer	1 x 30 mL	
TMB Substrate	1 x 12 mL	
Stop Solution	1 x 12 mL	

5. Precautions

- Read instructions fully prior to beginning use of the assay kit.
- Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
- Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
- For information on hazardous substances included in the kit refer to the Material Safety Data Sheet (MSDS).
- Kit cannot be used beyond the expiration date on the label.

6. Required Materials Not Supplied

- Microplate reader capable of reading absorbance at 450 nm.
- Automated plate washer (optional).
- Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
- Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
- New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
- Absorbent paper or paper toweling.
- Distilled or deionized ultrapure water.
- 37°C Incubator (optional)

7. Technical Application Tips

- Do not mix or substitute components from other kits.
- To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
- Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.

- Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.
- Replicate wells are recommended for standards and samples.
- Cover microplate while incubating to prevent evaporation.
- Do not allow the microplate wells dry at any point during the assay procedure.
- Do not reuse tips or tube to prevent cross contamination.
- Avoid bubbles or foaming when pipetting, mixing, or reconstituting.
- Completely remove of all liquids when washing to prevent cross contamination.
- Prepare reagents immediately prior to use and do not store, except for the top standard.
- For optimal results for inter- and intra-assay consistency, equilibrate all materials (except for standards) to 37°C prior to performing assay and perform all incubations at 37°C.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.
- Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
- Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
- Samples that are hemolytic, lipemic, or contain precipitates, fibrin strands or bilirubin may cause inaccurate results due to interfering factors.
- TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

8. Reagent Preparation

- Equilibrate all materials to room temperature prior to use and prepare immediately prior to use.
- Prepare the EN2 Standard no greater than 2 hours prior to performing experiment. A fresh lyophilized vial should be reconstituted for each experiment. Standards should be held on ice until use in the experiment.

8.1 Human EN2 Assay Standards

8.1.1 Reconstitute the EN2 **Standard** by adding 1 mL of 1X PBS directly to the vial to achieve **Standard #1**. Close, and invert several times to dissolve. Do not vortex. Allow the vial to sit at room temperature for 10 minutes before use.

8.1.2 Prepare a set of serially diluted standards as follows:

8.1.2.1 Label tubes 1 - 7 with each respective standard number.

8.1.2.2 Add the entire contents of the reconstituted standard to Tube #1.

8.1.2.3 Add 250 µL of **Assay Diluent A** to the remaining tubes.

8.1.2.4 Prepare **Standard #2** by adding 250 µL of **Standard #1** from Tube #1 to Tube #2. Vortex briefly to mix.

8.1.2.5 Prepare **Standard #3** by adding 250 µL of **Standard #2** from Tube #2 to Tube #3. Vortex briefly to mix.

8.1.2.6 Prepare further serial dilutions in the remaining tubes. Reference the table below as a guide for serial dilution scheme.

8.1.2.7 The last tube is a blank standard (only **Assay Diluent A**), which should be included with every experiment.

Standard Number (Tube)	Standard To Dilute (ng/mL)	Volume Standard to Dilute (µL)	Volume Assay Diluent (µL)	Total Volume (µL)	Final Concentration (ng/mL)
1	30 ng/mL Reconstituted EN2 Standard	NA	NA	1,000	30
2	30	250	250	500	15
3	15	250	250	500	7.5
4	7.5	250	250	500	3.75
5	3.75	250	250	500	1.87
6	1.87	250	250	500	0.94
7	0.94	250	250	500	0.47
8	NA	0	250	500	0.0 (Blank)

8.2 Biotinylated anti-EN2 Antibody Working Solution

8.2.1 For each 96 well plate, prepare 11 mL of Biotinylated anti-EN2 Antibody Working Solution by diluting Human EN2 Biotin Detection Antibody with Assay Diluent A at a ratio suggested by COA.

8.3 HRP-Streptavidin Working Solution

8.3.1 For each 96 well plate, prepare **HRP-Streptavidin Working Solution** by diluting the HRP-streptavidin **Assay Diluent**. **A** at a ratio suggested by COA.

8.4 1X Wash Buffer

8.4.1 If crystals have formed in the **20X Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

8.4.2 Add the entire 30ml contents of the **20X Wash Buffer** bottle to 570ml of ultra-pure water in a clean bottle.

8.4.3 Mix gently and thoroughly by magnetic stirrer. **Avoid foaming or bubbles.**

8.4.4 Hold the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 2 weeks. Do not freeze.

8.5 Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Any unused well strips should be sealed in the original packaging and stored at 4°C with desiccant.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation

9.1 Sample Preparation and Storage

- Store samples to be assayed at 2-8°C for 24 hours prior to being assayed.
- For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.
- This assay is intended for serum, plasma and other biological fluids. The sample preparation protocols below have been provided for your reference:

    - **Serum** - Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

    - **Plasma** - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

    - **Tissue Homogenates** – Rinse 100 mg of tissue with 1X PBS, then homogenize in 1 mL of 1X PBS and stored overnight at -20°C. Perform two freeze-thaw cycles to break the cell membranes, then centrifuge the homogenates for 5 minutes at 5,000 x g, 2-8°C. Remove the supernatant and assay immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

    - **Cell culture supernatants** – Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Note: Samples that are hemolytic, lipemic, or contain precipitates, fibrin strands or bilirubin may cause inaccurate results due to interfering factors

9.2 Sample Dilution

Target protein concentration must be estimated, and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting saturation should be further diluted.

- Dilute cell culture supernatant or recombinant protein samples using **Assay Diluent A**. Special assay diluent (not included) may be required for serum, plasma and tissue homogenates samples.
- Mix diluted samples gently and thoroughly.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.

10. Assay Procedure

Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

10.1 Add 100 µL of the serially diluted EN2 **Standards**, or diluted samples or blank into wells of the **Anti-EN2 Microplate**. At least two replicates of each standard, sample or blank is recommended.

10.2 Seal the plate and incubate for 1 hour at room temperature.

10.3 Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.5 Wash plate 5 times with **1X Wash Buffer** as follows:

- **10.5.1** Add 300 µL of **1X Wash Buffer** to each assay well.
- **10.5.2** Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
- **10.5.3** Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
- **10.5.4** Repeat steps 10.5.1 through 10.5.3 **four** more times.

10.6 Add 100 uL Biotin-anti-EN2 antibody to each well and incubated at room temperature for 1 hour.

10.7 Wash the plate 5 times as step 10.5.

10.8 Add 100 µL **HRP-Streptavidin Working Solution** to each well.

10.9 Cover the plate with plate seal and incubate for 60 minutes at room temperature.

10.10 Discard the liquid in the wells by aspiration.

10.11 Wash plate **5 times** as in Step 10.5

10.12 Add 100 µL of **TMB Substrate** to each well and incubate **for 5-30 minutes in the dark.** Wells should change to gradations of blue. If the color is too deep, reduce the incubation time.

(NOTE: TMB Substrate must be pre-warmed to room temperature before adding to the plate. **Incubation time must be determined by the user.** Optimal development can be visualized by blue shading in the top four standard wells. The blank (final standard point) should be faint blue to clear.)

10.13 Add 50 µL of **Stop Solution** to each well. Well color should change to yellow immediately. Add the **Stop Solution** in the same well order as done for the **TMB Substrate.**

10.14 Read the O.D. absorbance at 450 nm with a standard microplate reader within 30 minutes of stopping the reaction in step 10.11

11. Calculation of Results

For analysis of the assay results, calculate the **Relative OD_{450}** for each test or standard well as follows:

$$(\text{Relative } OD_{450}) = (\text{Well } OD_{450}) - (\text{Mean Blank Well } OD_{450})$$

The standard curve is generated by plotting the mean replicate **Relative OD_{450}** of each standard serial dilution point vs. the respective standard concentration. The {target} concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD_{450}** against the standard curve. This is best achieved using curve fitting software.

Note: If wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

Note: If the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

12. Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

USA

Aviva Systems Biology, Corp.
7700 Ronson Rd, Ste 100
San Diego, CA 92111

Phone: 858-552-6979
Toll Free: 888-880-0001
Fax: 858-552-6975

Technical support: techsupport@avivasysbio.com

China

Beijing AVIVA Systems Biology
6th Floor, B Building, Kaichi Tower
#A-2 Jinfu Road.
Daxing Industrial Development Zone
Beijing, 102600, CHINA

Phone: (86)10-60214720
Fax: (86)10-60214722
E-mail: support@avivasysbio.com.cn

中国地址：北京大兴工业开发区金辅路甲 2 号凯驰大厦 B 座 6 层 (102600)
电话: 010-60214720/21
传真: 010-60214722

产品售前咨询及销售: sales@avivasysbio.com.cn
售后及技术支持: support@avivasysbio.com.cn