ZC3H12A ELISA Kit (Human) (OKWB00161)

Lot# KE0572

Instructions for Use

For the quantitative measurement of ZC3H12A in serum, plasma, tissue homogenates and other biological fluids.

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

This product is intended for research use only.

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1. Background

Principle

Aviva Systems Biology ZC3H12A ELISA Kit (Human) (OKWB00161) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific for ZC3H12A has been pre-coated onto a 96-well plate (12 x 8 Well Strips). Standards or test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for ZC3H12A is added, incubated and followed by washing. Avidin-Peroxidase Conjugate is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is produced through the addition of TMB substrate which is catalyzed by HRP generating a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm is quantitatively proportional to the amount of sample ZC3H12A captured in the well.

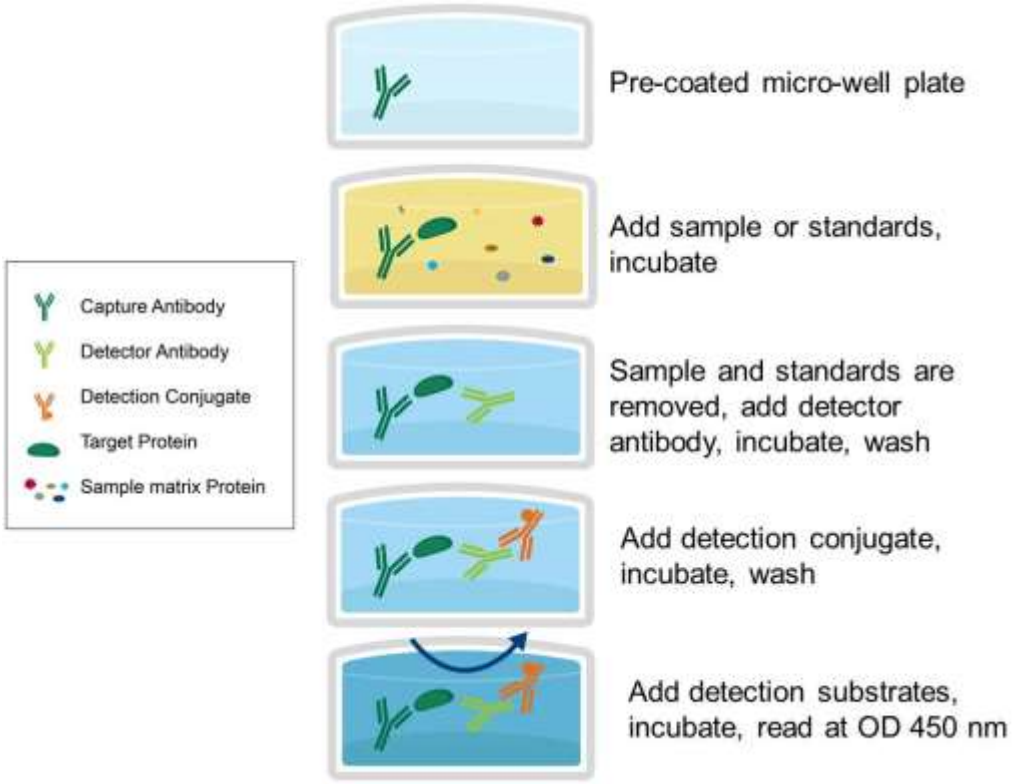
Target Background

Endoribonuclease involved in various biological functions such as cellular inflammatory response and immune homeostasis, glial differentiation of neuroprogenitor cells, cell death of cardiomyocytes, adipogenesis and angiogenesis. Functions as an endoribonuclease involved in mRNA decay. Modulates the inflammatory response by promoting the degradation of a set of translationally active cytokine-induced inflammation-related mRNAs, such as IL6 and IL12B, during the early phase of inflammation. Prevents aberrant T-cell-mediated immune reaction by degradation of multiple mRNAs controlling T-cell activation, such as those encoding cytokines (IL6 and IL2), cell surface receptors (ICOS, TNFRSF4 and TNFR2) and transcription factor (REL).

General Specifications

General Specifications	
Range	3.9 - 250 pg/mL
LOD	< 2.34 pg/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Human Endoribonuclease ZC3H12A UniProt ID: Q5D1E8 GeneID: 80149 Target Alias: dJ423B22.1, FLJ23231, MCP-induced protein 1, MCPIP, MCPIP1, Ribonuclease ZC3H12A, RP3-423B22.1, Zinc finger CCCH domain-containing protein 12A
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

• Open kit immediately upon receipt. Store components at -20°C (NOTE: exceptions below) for 6 months or until expiration date. Avoid any freeze/thaw cycles.

4. Kit Components

• The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
ZC3H12A Microplate	96 Wells (12 x 8 Well strips)	-20°C for 6 months
ZC3H12A Lyophilized Standard	2 x 250 pg	
100X Biotinylated ZC3H12A Detector Antibody	1 x 120 µL	4°C for 6 months
100X Streptavidin-HRP Conjugate	1 x 120 µL	
Sample Diluent	1 x 20 mL	
Detector Antibody Diluent	1 x 10 mL	
SABC Dilution Buffer	1 x 10 mL	
25X Wash Buffer	1 x 30 mL	
Stop Solution	1 x 10 mL	
TMB Substrate	1 x 10 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 please 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 causing 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, with the exception of the top standard.

• Equilibrate all materials to ambient room temperature prior to use (standards exception).

• For optimal results for inter- and intra-assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception) and perform all incubations at 37°C.

• Pipetting less than 1 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 containing bilirubin, precipitates or fibrin strands or are hemolytic or lipemic might 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 use immediately.

8.1 ZC3H12A Assay Standards

8.1.1 Prepare the ZC3H12A standards no greater than 2 hours prior to performing experiment. Standards should be held on ice until use in the experiment.

8.1.2 Reconstitute one vial of the provided **250 pg Lyophilized Standard** for each experiment. Prepare a stock **250 pg/mL Standard** by reconstituting one tube of **Lyophilized Standard** as follows:

8.1.2.1 Gently spin or tap the vial at 6,000 – 10,000 rpm for 30 seconds to collect all material at the bottom.

8.1.2.2 Add 1 mL of **Sample Diluent** to the vial.

8.1.2.3 Seal the vial then mix gently and thoroughly.

8.1.2.4 Leave the vial at ambient temperature for 15 minutes.

8.1.3 Prepare a set of serially diluted standards as follows:

8.1.3.1 Label tubes with numbers 2 – 8.

8.1.3.2 Use the undiluted **250 pg/mL Standard** from step 8.1.2 as the high standard point (Tube #1).

8.1.3.3 Add 300 µL of **Sample Diluent** to Tube #'s 2 – 8.

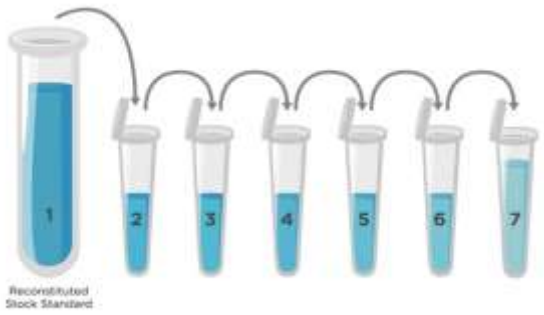
8.1.3.4 Prepare **Standard #2** by adding 300 µL of **250 pg/mL Standard** (Tube #1) to Tube #2. Mix gently and thoroughly.

8.1.3.5 Prepare **Standard #3** by adding 300 µL of **Standard #2** from Tube #2 to Tube #3. Mix gently and thoroughly.

8.1.3.6 Prepare further serial dilutions through Tube #7. Reference the table below as a guide for serial dilution scheme.

8.1.3.7 Tube #8 is a blank standard (only **Sample Diluent**), which should be included with every experiment.

Standard Number (Tube)	Standard To Dilute	Volume Standard to Dilute (µL)	Volume Sample Diluent (µL)	Total Volume (µL)	Final Concentration
1	250 pg/mL Reconstituted Standard	NA	1,000	1,000	250 pg/mL
2	250 pg/mL	300	300	600	125 pg/mL
3	125 pg/mL	300	300	600	62.5 pg/mL
4	62.5 pg/mL	300	300	600	31.2 pg/mL
5	31.2 pg/mL	300	300	600	15.6 pg/mL
6	15.6 pg/mL	300	300	600	7.8 pg/mL
7	7.8 pg/mL	300	300	600	3.9 pg/mL
8	NA	0	300	300	0.0 (Blank)



8.2 1X Biotinylated ZC3H12A Detector Antibody

8.2.1 Prepare the **1X Biotinylated ZC3H12A Detector Antibody** immediately prior to use by diluting the **100X Biotinylated ZC3H12A Detector Antibody** 1:100 with **Detector Antibody Diluent**.

8.2.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Biotinylated ZC3H12A Detector Antibody** to 990 µL **Detector Antibody Diluent**.

8.2.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in the procedure. Do not store at 1X concentration for future use.

8.3 1X HRP-Streptavidin Conjugate (SABC)

8.3.1 Prepare the **1X HRP-Streptavidin Conjugate** immediately prior to use by diluting the **100X HRP-Streptavidin Conjugate** 1:100 with **SABC Dilution Buffer**.

8.3.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X HRP-Streptavidin Conjugate** to 990 µL **SABC Dilution Buffer**.

8.3.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.4 1X Wash Buffer

8.4.1 If crystals have formed in the **25X Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

8.4.2 Add the entire 30 mL contents of the **25X Wash Buffer** bottle to 720 mL of ultra-pure water to a clean > 1,000 mL bottle or other vessel.

8.4.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

8.4.4 Store 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 1 week. Do not freeze.

8.5 Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
- 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 being assayed.
- For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.
- Samples not indicated in the manual must be tested to determine if the kit is valid.
- Prepare samples as follows:

  - **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes 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 1X PBS, homogenize in 1 mL of 1X PBS then store 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.
  - **Other biological fluids** – Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

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 samples using **Sample Diluent**.
- Mix diluted samples gently and thoroughly.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.

10. Assay Procedure

• Equilibrate all reagents (except TMB) and materials to ambient room temperature prior to use in the procedure.

• Equilibrate TMB Substrate for 30 minutes at 37°C.

10.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch. Wash plate 2 times as follows:

10.2 Wash plate 2 times with **1X Wash Buffer** as follows:

10.2.1 Add 300 µL of **1X Wash Buffer** to each assay well.

10.2.2 Incubate for 1 minute.

10.2.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.2.4 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.

10.2.5 Repeat steps 10.2.1 through 10.2.5.

10.3 Add 100 µL of serially titrated standards, diluted samples or blank into wells of the **ZC3H12A Microplate**. At least two replicates of each standard, sample or blank is recommended.

10.4 Cover the plate with the plate sealer and incubate at 37°C for 90 minutes.

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

10.6 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.

10.7 Wash plate **2 times** with **1X Wash Buffer** as in step 10.2.

10.8 Add 100 µL of prepared **1X Biotinylated ZC3H12ADetector Antibody** to each well.

10.9 Cover with the plate sealer and incubate at 37°C for 60 minutes.

10.10 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.11 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.

10.12 Wash plate **3 times** with **1X Wash Buffer** as in step 10.2.

10.13 Add 100 µL of prepared **1X HRP-Streptavidin Conjugate** into each well, cover with plate sealer and incubate at 37°C for 30 minutes.

10.14 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.15 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.

10.16 Wash plate **5 times** with **1X Wash Buffer** as in Step 10.2.

10.17 Add 90 µL of **TMB Substrate** to each well, cover with plate sealer and incubate at 37°C **in the dark** for 10-20 minutes. Wells should change to gradations of blue. If the color is too deep, reduce the incubation time.

(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)

10.18 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**.

10.19 Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 10.18. If wavelength correction is available, set to 540 nm or 570 nm.

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 **ZC3H12A** 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. Typical Expected Data

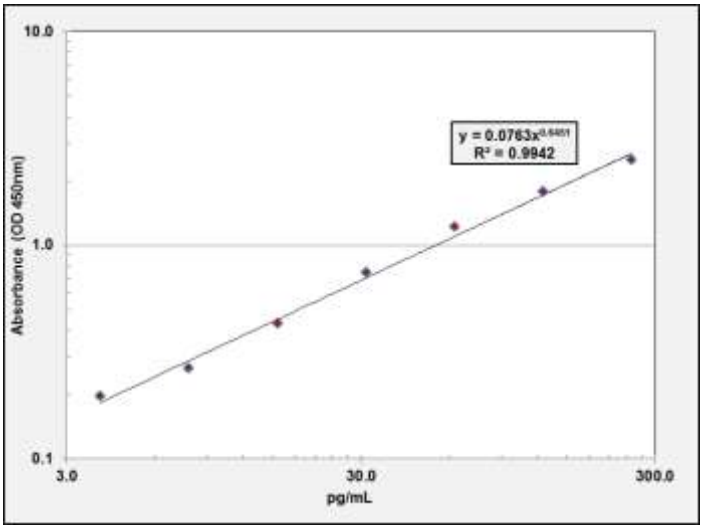
12.1 Reproducibility

Three samples concentrations were measured in replicate within an assay plate and across replicate assays to assess Intra- and Mean Inter-Assay Precision.

Mean Intra-Assay Precision - ≤ 10 %

Mean Inter-Assay Precision - ≤ 12%

12.2 Typical standard curve. This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.



pg/mL	Absorbance		Mean Absorbance	Blank Subtracted
	Rep 1	Rep 2		
250	2.502	2.626	2.564	2.488
125	1.873	1.827	1.850	1.774
62.5	1.251	1.315	1.283	1.207
31.3	0.802	0.826	0.814	0.738
15.6	0.511	0.487	0.499	0.423
7.8	0.331	0.347	0.339	0.263
3.9	0.263	0.277	0.270	0.194
Blank	0.077	0.075	0.076	NA

12.3 Linearity

Kit linearity evaluated by testing serially diluted samples containing known concentrations of ZC3H12A. Results are expressed as the percentage of the expected concentration measurement.

Sample Type	Dilution Level		
	1:2	1:4	1:8
serum(n=5)	96-105%	89-105%	85-97%
EDTA plasma(n=5)	82-97%	85-95%	83-96%
heparin plasma(n=5)	85-100%	80-100%	80-95%

12.4 Recovery

The following matrices were spiked to known concentrations using recombinant ZC3H12A. Recovery is expressed as the percentage of the expected concentration measurement.

Sample Type	Recovery Range (%)	Average (%)
serum(n=5)	85-103	92
EDTA plasma(n=5)	87-105	93
heparin plasma(n=5)	94-103	99

13. Technical Resources

Technical Support:

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

USA

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