IP3 ELISA Kit (OKCD02270)

Lot# BJ08162022

Instructions for Use

For the quantitative measurement of IP3 in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants 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 IP3 ELISA Kit (OKCD02270) is based on a competitive enzyme immuno assay technique. The microtiter well-plate in this kit has been pre-coated with an anti-IP3 antibody. Sample or standards are added to the wells along with a fixed quantity of biotinylated IP3 and incubated. The IP3 found in the sample or standards competes with the biotinylated IP3 for limited binding sites on the immobilized anti-IP3 antibody. Wells are washed and Avidin-HRP conjugate is added, incubated then washed. An enzymatic reaction is then produced through the addition of TMB substrate which is catalyzed by the immobilized HRP to generate a blue color product that changes yellow after adding acidic stop solution. The density of yellow coloration is measured by reading the absorbance at 450 nm which is quantitatively proportional to the amount of biotinylated IP3 captured in the well and inversely proportional to the amount of IP3 which was contained in the sample or standard.

Target Background

Intracellular messenger formed by the action of phospholipase C on phosphatidylinositol 4,5-bisphosphate, which is one of the phospholipids that make up the cell membrane. Inositol 1,4,5-trisphosphate is released into the cytoplasm where it releases calcium ions from internal stores within the cell's endoplasmic reticulum. These calcium ions stimulate the activity of B kinase or calmodulin.

General Specifications

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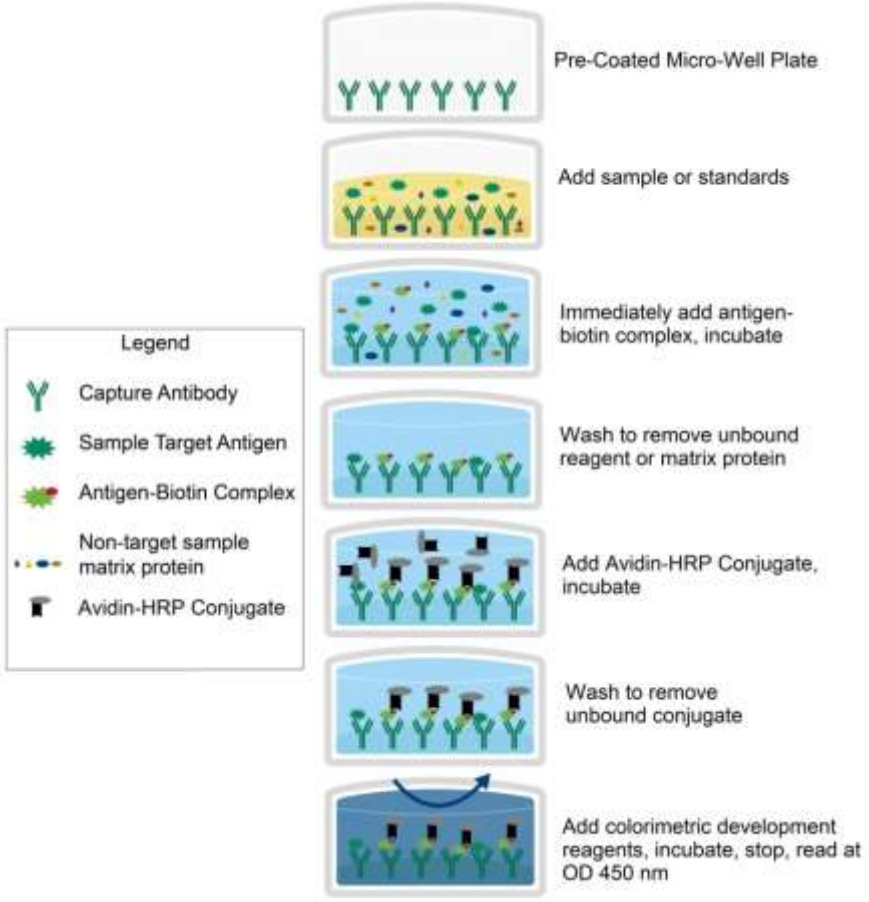
Range	123.5-10,000 pg/mL
LOD	< 45.4 pg/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Inositol Triphosphate CAS: 85166-31-0 PubChem: 439456 Target Alias: Inositol 1,4,5-trisphosphate; 1,4,5-Insp3; D-myo-Inositol 1,4,5-trisphosphate; InsP3; 1D-myo-Inositol 1,4,5-trisphosphate; D-myo-inositol-1,4,5-triphosphate
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
Anti-IP3 Microplate	96 Wells (12 x 8 Well strips)	-20°C for 6 months
IP3 Lyophilized Standard	2 x 10 ng	
100X IP3-Biotin Complex	1 x 120 µL	
100X Avidin-HRP Conjugate	1 x 120 µL	
Standard Diluent	1 x 20 mL	4°C for 6 months
Biotin Complex Diluent	1 x 12 mL	
Conjugate Diluent	1 x 12 mL	
30X Wash Buffer	1 x 20 mL	
TMB Substrate	1 x 9 mL	
Stop Solution	1 x 6 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 in inter- 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 precipitates or fibrin strands or which are hemolytic of 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 IP3 Assay Standards

8.1.1 Prepare the IP3 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 10 ng **Lyophilized IP3 Standard** for each experiment. Prepare a stock 10,000 pg/mL Standard by reconstituting one tube of **Lyophilized IP3 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 **Standard 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 – 6.

8.1.3.2 Use the undiluted 10,000 pg/mL **IP3 Standard** from step 8.1.2 as the high standard point (Tube #1).

8.1.3.3 Add **600 µL** of **Standard Diluent** to Tube #'s 2 – 6.

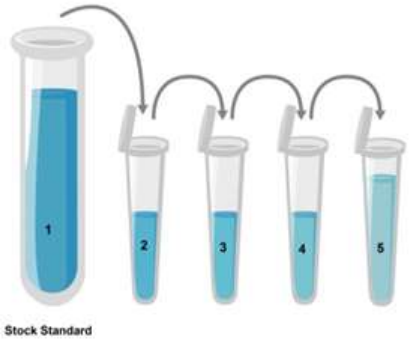
8.1.3.4 Prepare **Standard #2** by adding 300 µL of 10,000 pg/mL **IP3** (Tube #1) to Tube #2. Mix gently and thoroughly.

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 #6. Reference the table below as a guide for serial dilution scheme.

8.1.3.7 Tube #6 is a blank standard (only **Standard Diluent**), which should be included with every experiment.

Standard Number (Tube)	Standard To Dilute	Volume Standard to Dilute (µL)	Volume Standard Diluent (µL)	Total Volume (µL)	Final Concentration
Lyophilized Stock (1)	Lyophilized Stock	NA	1,000	1,000	10,000 pg/mL
2	10,000 pg/mL	300	600	900	3,333.3 pg/mL
3	3,333.3 pg/mL	300	600	900	1,111.1 pg/mL
4	1,111.1 pg/mL	300	600	900	370.4 pg/mL
5	370.4 pg/mL	300	600	900	123.5 pg/mL
6	NA	0	600	600	0.0 (Blank)



8.2 1X IP3-Biotin Complex

8.2.1 Prepare the **1X IP3-Biotin Complex** immediately prior to use by diluting the **100X IP3-Biotin Complex** 1:100 with **Biotin Complex 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 IP3-Biotin Complex** to 990 µL **Biotin Complex Diluent**.

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

8.3 1X Avidin-HRP Conjugate

8.3.1 Prepare the **1X Avidin-HRP Conjugate** immediately prior to use by diluting the **100X Avidin-HRP Conjugate**1:100 with **Conjugate Diluent** as follows.

8.3.2 Briefly and gently mix the **100X Avidin-HRP Conjugate** prior to pipetting.

8.3.3 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL **1X Avidin-HRP Conjugate** by adding 10 µL of **100X Avidin-HRP Conjugate** to 990 µL **Conjugate Diluent**.

8.3.4 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure.

8.4 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.

8.5 1X Wash Buffer

8.5.1 If crystals have formed in the **30X Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

8.5.2 Add the entire 20 mL contents of the **30X Wash Buffer** bottle to 580 mL of ultra-pure water to a clean > 1,000 mL bottle or other vessel.

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

8.5.4 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.

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 tissue with 1X PBS to remove excess blood, homogenize in 20 mL of 1X PBS and store overnight at ≤ -20°C. Perform two freeze-thaw cycles to break the cell membranes, then centrifuge homogenates for 5 minutes at 5,000 x g. Remove the supernatant and assay immediately or aliquot and store at ≤ -20°C.
- **Cell culture supernatants and 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.
- **Cell Lysates** - Cells need to be lysed before assaying according to the following directions. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000 x g for 5 minutes (suspension cells can be collected by centrifugation directly). Wash cells three times in cold PBS.
  3. Resuspend cells in fresh lysis buffer with concentration of 10^7 cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified.
  4. Centrifuge at 1,500 x g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤ -20°C.

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.

- Dilute samples using **Standard Diluent**.
- Mix diluted samples gently and thoroughly.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.
- Optimal dilution must be determined by the user according to their specific samples.

10. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.
• For optimal control of small potential variations in micro well-plate and day to day ambient temperature fluctuations, equilibrate all reagents prior to use and perform all incubation steps at 37°C.

10.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

10.2 Retain at least one well as an absolute Blank without any samples or reagents.

10.3 Add 50 µL of serially titrated standards, diluted samples or blank into wells of the **Anti-IP3 Microplate**. At least two replicates of each standard, sample or blank is recommended.

10.4 Immediately add 50 µL of **1X IP3-Biotin Complex** to each well (excluding absolute Blank).

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

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

10.7 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

10.8 Wash plate three times with **1X Wash Buffer** as follows:

10.8.1 Add 350 µL of **1X Wash Buffer** to each assay well.

10.8.2 Incubate for 2 minutes.

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

10.8.4 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.

10.8.5 Repeat steps 10.8.1 through 10.8.4 **two** more times.

10.9 Add 100 µL of **1X Avidin-HRP Conjugate** to each well.

10.10 Cover the plate with the plate sealer and incubate at 37°C for 30 minutes.

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

10.12 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.

10.13 Repeat wash as in **step 10.8.**

10.14 Add 90 µL of **TMB Substrate** to each well 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 based on the standard, adjust incubation times.

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

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

10.16 Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 10.15. 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 IP3 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 are 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

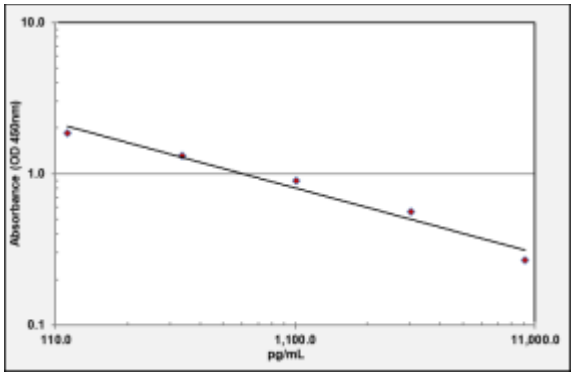
12.1 Reproducibility

Intra-assay reproducibility was evaluated with 20 replicates of 3 samples representing low, middle and high level target. Inter-assay reproducibility was evaluated with 3 samples representing low, middle and high level target using 8 replicates on each of 3 plates.

Sample	Intra-Assay			Inter-Assay		
	1	2	3	1	2	3
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/ml)	315.46	1012.65	3386.74	315.29	1013.12	3387.53
SD	14.511	52.658	189.657	14.819	53.695	193.089
CV (%)	4.6	5.2	5.6	4.7	5.3	5.7

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	Log of concentration
	Rep 1	Rep 2		
10,000	0.321	0.323	0.322	4.000
3,333	0.615	0.611	0.613	3.523
1111.1	0.949	0.955	0.952	3.046
370.4	1.368	1.362	1.365	2.569
123.5	1.907	1.899	1.903	2.092

12.3 Recovery

Matrices were spiked with IP3 and recovery rates were calculated by comparing the measured values to the expected concentrations.

Matrix	Recovery Range (%)	Mean Recover (%)
serum(n=5)	86 - 96	90
EDTA plasma(n=5)	80 - 94	87
heparin plasma(n=5)	78 - 107	100

12.4 Linearity

Linearity of the kit was evaluated by spiking IP3 into matrices, serially diluted and measured. Observed values were compared to the expected measurements.

Matrix	1:2	1:4	1:8	1:16
serum(n=5)	85-98%	98-109%	79-101%	84-95%
EDTA plasma(n=5)	95-102%	78-92%	88-105%	86-107%
heparin plasma(n=5)	80-96%	90-101%	83-97%	81-103%

13. Technical Resources

Technical Support:

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

USA

Aviva Systems Biology, Corp.
10211 Pacific Mesa Blvd, Ste 401
San Diego, CA 92121

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