Penicillin G ELISA Kit (OKAO00124)

Lot# KH0777

Instruction for Use

For the quantitative detection of Penicillin G (benzyl penicillin) concentration in tissue (chicken, duck, porcine meat, liver, shrimp, fish. etc), egg and milk.

Variation between lots 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 Penicillin G ELISA Kit (OKAO00124) is based on standard competitive inhibition enzyme-linked immuno-sorbent assay technology. Penicillin G has been pre-coated onto a 96-wellplate (12 x 8 Well Strips). Standards or test samples are added to the wells along with an HRP Conjugated antibody, then incubated. The Penicillin G in the sample and the coupling antigens pre-coated on the microwell stripes compete for the enzyme conjugate anti-Penicillin G antibodies. Wells are washed and detection substrates are added and incubated. An enzymatic reaction is produced through the addition of TMB 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 which is quantitatively proportional to the amount of detector antibody bound in the well and inversely proportional to the amount of Penicillin G in the sample.

Background

Benzyl penicillin (INN, AAN, BAN), commonly known as penicillin G, (USAN) is a narrow spectrum penicillin antibiotic that is given intravenously or intramuscularly as a treatment for syphilis, meningitis, endocarditis, pneumonia, lung abscesses and septicaemia in children. Penicillin G is typically given by injection parenterally, bypassing the intestines, because it is unstable in the highly acidic stomach. Because the drug is given parenterally, higher tissue concentrations of penicillin G can be achieved than is possible with phenoxymethylpenicillin. These higher concentrations translate to increased antibacterial activity. It is on the World Health Organization's List of Essential Medicines, a list of the most important medications needed in a basic health system.

General Specifications

General Specifications	
Range	0.05 – 4.05 ppb
LOD	< 0.05 ppb (Derived by linear regression of OD450 of the Mean Blank + 2xSD)
Specificity	Penicillin G CAS#: 69-57-8 PubChem#: 23668834 Target Alias: benzyl penicillin; Penicillin G sodium; Benzylpenicillin sodium; Penicillin G sodium salt; Crystapen; 69-57-8; BENZYLPENICILLIN SODIUM SALT
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Storage and Stability

• Upon receipt store kit at 4°C. Do not use past expiration date.

3. Kit Components

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

Description	Quantity	Storage Conditions
Penicillin G Microplate	96 Wells (12 x 8 Well strips)	Store at 4°C Do not use past expiration date
Penicillin G Standards	6 x 1 mL	
11X HRP-Conjugate	1 x 700 µL	
HRP Conjugate Diluent	1 x 7 mL	
Substrate A	1 x 7 mL	
Substrate B	1 x 7 mL	
Sample Diluent	1 x 20 mL	
20X Wash Buffer	1 x 20 mL	
Stop Solution	1 x 7 mL	

4. 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.

• Heater water bath

• Centrifuge

• Reagents: HCl (approx. 36.5%), deionized water.

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. 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 precipitates, fibrin strands or bilirubin, 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.

• To minimize influences on the assay performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

7. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use immediately.

7.1 Penicillin G Standards

7.1.1 Standards are provided at ready to use concentrations. The concentrations are as follows:

Standard 0	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5
0 ppb	0.05 ppb	0.15 ppb	0.45 ppb	1.35 ppb	4.05 ppb

7.2 1X Wash Buffer

7.2.1 Dilute 20 mL of the **20X Wash Buffer** with distilled or deionized water at 1:19 to 400 mL (or the required volume) immediately prior to use.

7.2.2 Mix gently and thoroughly. Do not store for future use.

7.3 1X HRP-Conjugate

7.3.1 Dilute 700 µL of the **11X HRP-Conjugate** with the **HRP-Conjugate Diluent** at 1:11 to 7.7 mL (or just to the required volume) for use.

7.3.2 Mix gently and thoroughly. Do not store for future use.

7.4 1M HCl solution

7.4.1 Dissolve 8.6 mL HCL (approx. 36.5%) in deionized water to 100 mL.

7.4.2 Mix gently and thoroughly. Do not store for future use.

7.5 Microplate Preparation

7.5.1 Micro-plates are provided ready to use and do not require rinsing or blocking.

7.5.2 Unused well strips should be returned to the original packaging, sealed and stored at 4°C.

7.5.3 Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

8. Sample Preparation

8.1 Sample Preparation and Storage

- Mix diluted samples gently and thoroughly.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.
- Untreated samples should be stored at -20°C.
- Prepared samples can be stable for 1 week at 4°C.

8.1.1 Tissue - (Diluted 10-Fold)

8.1.1.1 Homogenize the sample.

8.1.1.2 Weigh 1 ± 0.05 g of the homogenized sample, place into 10 mL centrifugal tube, add 4 mL deionized water, and shake thoroughly for 1 minute.

8.1.1.3 Centrifuge at above 3000 rpm at 25°C for 5 minutes.

8.1.1.4 Transfer 0.2 mL supernatant into a new centrifuge tube.

8.1.1.5 Add 0.2 mL of the **Sample Diluent** and mix properly for 10 seconds.

8.1.1.6 Take 50 µL of the lower layer for analysis.

8.1.2 Egg – (Diluted 10-Fold)

8.1.2.1 Homogenize the sample.

8.1.2.2 Weigh 1 ± 0.05 g of the homogenized sample, place into 10 mL centrifugal tube, add 4 mL deionized water and 150 µL **1M HCl solution**, and shake thoroughly for 1 minute.

8.1.2.3 Centrifuge at above 3000 rpm at 25°C for 5 minutes.

8.1.2.4 Transfer 0.2 mL supernatant into a new centrifuge tube.

8.1.2.5 Add 0.2 mL of the **Sample Diluent** and mix properly for 10 seconds.

8.1.2.6 Take 50 µL of the lower layer for analysis.

8.1.3 Milk – (Diluted 10-Fold)

8.1.3.1 Homogenize the sample.

8.1.3.2 Weigh 1 ± 0.05 g of the homogenized sample, place into 10 mL centrifugal tube, add 4 mL deionized water and 40 µL **1M HCl solution**, and shake thoroughly for 1 minute.

8.1.3.3 Centrifuge at above 3000 rpm at 25°C for 5 minutes.

8.1.3.4 Transfer 0.2 mL supernatant into a new centrifuge tube.

8.1.3.5 Add 0.2 mL of the **Sample Diluent** and mix properly for 10 seconds.

8.1.3.6 Take 50 µL of the lower layer for analysis.

9. Assay Procedure

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

• Temperature regulation for all procedures and incubations to 25°C is recommended for optimal reproducibility.

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

9.2 Add 50 µL of standards or samples into wells of the **Penicillin G Microplate**. At least two replicates of each standard, sample or blank is recommended.

9.3 Immediately add 50 µL of the **1X HRP-Conjugate** to each well.

9.4 Cover the plate with the plate sealer, gently mix and incubate for 30 minutes.

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

9.6 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.

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

9.7.1 Add 250 µL of **1X Wash Buffer** to each assay well.

9.7.2 Incubate for 30 seconds.

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

9.7.4 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.

9.7.5 Repeat steps 9.7.1 through 9.7.4 **four** more times.

9.8 Add 50 µL of prepared **Substrate A** and 50 µL of **Substrate B** to each well.

9.9 Gently mix the plate and incubate for 10-15 minutes **in the dark**.

9.10 Add 50 µL of **Stop Solution** to each well and mix gently. Well color should change to yellow immediately. Add the **Stop Solution** in the same well order as done for the substrate solutions.

9.11 Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 9.10. If wavelength correction is available, set to 630 nm.

10. Calculation of Results

For analysis of the assay results, first derive the **Percent Relative Absorbance** for each sample or standard by calculating the mean absorbance between replicate wells. This value is then divided by the mean replicate Blank (0 ppb) absorbance, then multiplying by 100%:

$$\text{Percent Relative Absorbance (Sample or Standard)} = \frac{\text{Mean Absorbance (Sample or Standard)}}{\text{Mean Blank Absorbance}} \text{ X } 100\%$$

A standard curve is generated by plotting the **Percent Relative Absorbance** of each standard serial dilution point vs. the respective standard concentration. The Penicillin G concentration contained in the samples can be interpolated by using linear regression of each sample **Percent Relative Absorbance** against the standard curve. This is best achieved using curve fitting software.

Note: If wavelength correction readings were available, subtract the readings at 630 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.

11. Typical Expected Data

11.1 Limit of Detection

Derived by linear regression of OD_{450} of the Mean Blank + 2xSD

Sample	Mean Recovery
Tissue	1 ppb
Egg	1 ppb
Milk	1 ppb

11.2 Reproducibility

Three samples of known concentrations were measured in 20 replicates within an assay plate to asses intra-assay reproducibility. Three samples of known concentrations were measured across 40 replicate assays inter-assay reproducibility:

Sample	Intra-Assay			Inter-Assay		
	1	2	3	1	2	3
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ppb)	0.336	0.625	1.029	0.412	0.732	1.158
SD	0.012	0.026	0.049	0.022	0.048	0.091
CV (%)	3.6	4.2	4.8	5.3	6.6	7.9

11.3 Cross-Reactivity Rate

Substance	Cross Reactivity Rate
Benzyl penicillin	100%

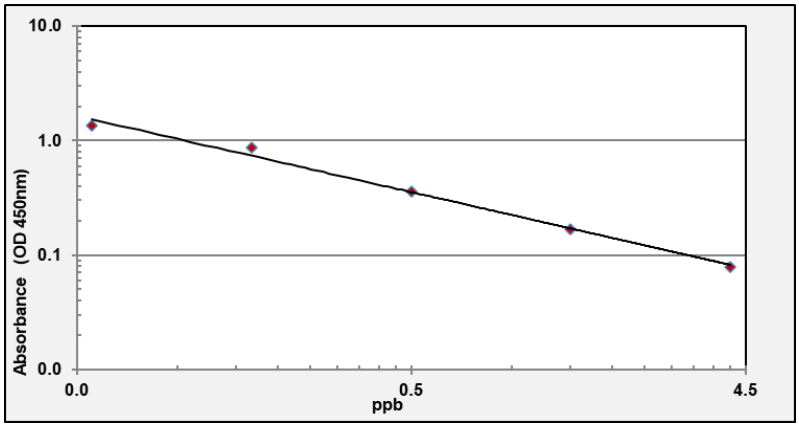
11.4 Recovery

The recovery of Penicillin G spiked at levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay as directed in the Sample Preparation section.

Sample	Number	Mean Recovery
Tissue	10	80% ± 15%
Egg	10	75% ± 20%
Milk	10	80% ± 15%

11.5 Typical Data

For convenience in result calculation, absorbance as abscissa and standard concentrations can be used as ordinate. The standard curve data provided in the manual is only for reference; experimenters should draw the standard curve based on their own data.



	ppb	Absorbance		Mean Absorbance	B/B0 (%)
		Rep 1	Rep 2		
1	4.05	0.079	0.063	0.071	100.0
2	1.35	0.167	0.141	0.154	73.8
3	0.45	0.362	0.374	0.368	47.7
4	0.15	0.870	0.874	0.872	20.1
5	0.05	1.354	1.344	1.349	8.4
6	0.00	1.855	1.801	1.828	3.9

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.
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