Datasheet for ABIN400587

# Ampicillin ELISA Kit

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## Overview

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| Quantity: | 96 tests |
| Target: | Ampicillin (AMP) |
| Reactivity: | Chemical |
| Method Type: | Competition ELISA |
| Application: | ELISA |

## Product Details

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| Purpose: | This test kit is based on the competitive enzyme immunoassay for the detection of Ampicillin in the sample. The coupling antigens are pre-coated on the micro-well stripes. The Ampicillin in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti-Ampicillin antibody. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with the Ampicillin in it. This value is compared to the standard curve and the Ampicillin concentration is subsequently obtained. |
| Analytical Method: | Qualitative and Quantitative |
| Detection Method: | Colorimetric |
| Characteristics: | Sensitivity: 0.5 ppb<br>Note: ppb=ng/mL or ng/g<br><br>Incubation Temperature: 25°C<br>Incubation Time: 30min-30min-15min<br>Detection limit: Tissue (method 1) about 3ppb, Tissue (method 2) about 10ppb. |

## Product Details

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| Components: | • Micro-well strips: 12 strips with 8 removable wells each<br>• Standard solution (1ml/bottle): 0ppb, 0.5ppb, 1.5ppb, 4.5ppb, 13.5ppb, 500ppb.<br>• Antibody working solution (7 mL)<br>• Enzyme conjugate (12 mL)<br>• Substrate A (7 mL)<br>• Substrate B (7 mL)<br>• Stop solution (7 mL)<br>• 20× concentrated washing buffer (30 mL)<br>• 2× concentrated redissolving solution (60 mL) |
| Material not included: | 1. Equipments: microplate reader (450nm, 630nm), rotary evaporator/nitrogen-drying device, homogenizer, oscillator, centrifuge (4000g and above), balance (a sensibility reciprocal of 0.01 g), measuring pipets, incubator (adjustable 25°C),timer<br>2. Micropipettors: single-channel 20~200 µL and 100~1000 µL, and eight-channel 30~300 µL<br>3. Reagents: Acetonitrile,deionized water, NaOH, n-hexane |

## Target Details

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| Target: | Ampicillin (AMP) |
| Alternative Name: | Ampicillin |
| Target Type: | Chemical |

## Application Details

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| Plate: | Pre-coated |
| Protocol: | **ELISA procedures**<br>1. Bring all reagents and micro-well strips to the room temperature (20-25°C).<br>2. Return all reagents to 2-8°C immediately after use.<br>3. The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in the procedures of ELISA.<br>4. For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.<br><br>**Operation procedures**<br>1. Take out all the necessary reagents from the kit and place at the room temperature (20 to 25°C) for at least 30 minutes. Note that each liquid reagent must be shaken to mix evenly before use.<br>2. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2-8°C, not frozen. |

3. Numbering: number the micro-wells according to samples and standard solution, each sample and standard solution should be performed in duplicate, record their positions.
4. Add 50μL of the sample or standard solution to separate duplicate wells, then add Antibody working solution, 50 μL each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 25°C at dark for 30 minutes.
5. Pour liquid out of microwell, add 250 μL/well of washing buffer for 15-30 seconds, repeat three to four times, then flap to dry (if there are the bubbles after flapping, cut them with the clean tips).
6. Add Enzyme Conjugate, 100ul each well, Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 25°C at dark for 30 minutes.
7. Pour liquid out of microwell, add 250 μL/well of washing buffer for 15-30 seconds, repeat three to four times, then flap to dry (if there are the bubbles after flapping, cut them with the clean tips).
8. Coloration: add 100 μL mixture of the substrate A and substrate B into each well (Note: mix Substrate A and Substrate B at 1:1, the mixture should be used in 10min, never use metal container or metal to stir the solution, otherwise the substrate may be invalid.). Mix gently by shaking the plate manually, and incubate at 25°C for 15 minutes at dark for coloration.
9. Determination: add 50 μL of the stop solution into each well (The substrate color from blue to yellow, it means the stop succeeds). Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value (Recommend to read the OD value at the dual-wavelength 450/630 nm within 5 minutes).

Sample Preparation:

**Sample pre-treatment**

Instructions The following points must be dealt with before the pre-treatment of any kind of sample:

1. Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents
2. Before the experiment, each experimental equipment must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.
   Solution preparation before sample pre-treatment:
   1. 0.1M NaOH solution(for tissue extracting): weigh 0.4g NaOH, add 100ml deionized water to dissolve and mix it evenly
   2. Acetonitrile-0.1M NaOH solution(for tissue extracting): take 84ml Acetonitrile, add 16ml 0.1M NaOH solution, mix it evenly.
   3. Redissolving solution: 1 part of 2× concentrated redissolving solution + 1 part of deionized water.
   4. Washing buffer: 1 part 20× concentrated washing buffer + 19 parts deionized water

**Tissue (method 1 ) (chicken, pork, fish, shrimp)**

1. Homogenize tissue samples with a homogenizer
2. Take 1.0 ± 0.05g homogenized tissue sample into 50ml Polystyrene centrifuge tube, add 4ml Acetonitrile-0.1M NaOH solution, vortex for 10min,centrifuge at 3000g at room temperature

(20 - 25 °C) for 10min

3. Take 1ml up-layer clear liquid into 10ml clean dry glass tube, blow to dry by nitrogen in 60 ℃ *water bath*
4. Dissolve the dry residues in 1 mL N-hexane, vortex for 30s, add 1 mL of the Redissolving solution, vortex for 30s, mix it evenly, then transfer it to a 2ml Polystyrene centrifuge tube, centrifuge at 3000g at room temperature (20-25°C) for 5 min
5. Remove the up-layer organic phase, take 50 µL down-layer liquid for analysis.
   **Fold of dilution of the sample: 4**

**Tissue (method 2 ) (chicken, pork, fish, shrimp)**

1. Homogenize tissue samples with a homogenizer
2. Take 1.0 ± 0.05g homogenized tissue sample into 50ml Polystyrene centrifuge tube, add 4ml Acetonitrile-0.1M NaOH solution, vortex for 10min,centrifuge at 3000g at room temperature (20 - 25 °C) for 10min
3. Take 1mll up-layer clear liquid into 10ml clean dry glass tube, blow to dry by nitrogen in 60℃ *water bath*
4. Dissolve the dry residues in 1 mL N-hexane, vortex for 30s, add 1 mL of the Redissolving solution, vortex for 30s, mix it evenly, then transfer it to a 2ml Polystyrene centrifuge tube, centrifuge at 3000g at room temperature (20-25°C) for 5 min
5. Remove the up-layer organic phase, take down-layer water phase, dilute with Redissolving solution at 1:4(50ul down-layer water phase+200ul Redissolving solution ).
6. Take 50ul for analysis.
   **Fold of dilution of the sample: 20**

Calculation of Results:

**Quantitative determination**

The mean values of the absorbance values is equivalent to the percentage of the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

Percentage of absorbance value = (B/B0)×100%

B—the average (double wells) OD value of the sample or the standard solution

B0—the average OD value of the 0 ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solutions and the semilogarithm values of the Ampicillin standard solutions (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, thus finally obtainingAmpicillin

## Application Details

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| | concentration in the sample. |
| Restrictions: | For Research Use only |

## Handling

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| Handling Advice: | **Precautions**<br>1. The room temperature below 25°C or the temperature of the reagents and the samples being not returned to the room temperature (20-25°C) will lead to a lower standard OD value.<br>2. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility.<br>3. Mix every reagent and reaction mixture evenly and wash the microplate thoroughly, otherwise there will be the undesirable reproducibility.<br>4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.<br>5. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light.<br>6. Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use.<br>7. Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of the 0 standard solution of less than 0.5 indicates its degeneration.<br>8. The optimum reaction temperature is 25 *℃, and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.* |
| Storage: | 4 °C |
| Storage Comment: | Store at 2-8 *℃, not frozen.* |