Datasheet for ABIN400596

Gentamicin ELISA Kit

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Overview

Quantity:	96 tests
Target:	Gentamicin (GEN)
Reactivity:	Chemical
Method Type:	Competition ELISA
Application:	ELISA

Product Details

Purpose:	This test kit is based on the competitive enzyme immunoassay for the detection of Gentamicin in the sample. The coupling antigen is pre-coated on the micro-well stripes. The Gentamicin in the sample and pre-coated coupling antigen on the micro-well stripes compete for the anti-Gentamicin 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 Gentamicin in it. The value is compared to the standard curve and the Gentamicin concentration is subsequently obtained.
Analytical Method:	Qualitative and Quantitative
Detection Method:	Colorimetric
Components:	Micro-well strips: 12 strips with 8 removable wells each 6 standard solution (1 mL each): 0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb, Enzyme conjugate (7 mL) red cap, Antibody working solution (7 mL) blue cap, Substrate A solution (7 mL) white cap, Substrate B solution (7 mL) black cap, Stop solution (7 mL) yellow cap, 20 concentrated washing buffer (40 mL) white cap, 2 concentrated redissolving solution (50 mL) transparent cap
Material not included:	Equipments: microplate reader (450 nm / 630 nm), votex, centrifuge, homogenizer, measuring

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	pipets and balance (a sensibility reciprocal of 0.01 g) Micropipettors: single-channel 20-200 L and 100-1000 L, and multi-channel 250 L, Reagents: Na2HPO412H2O, NaH2PO42H2O

Target Details

Target:	Gentamicin (GEN)
Alternative Name:	Gentamicin (GEN Products)
Target Type:	Chemical

Application Details

Plate:	Pre-coated
Protocol:	**Sample pre-treatment:** Instructions The following points must be dealt with before the pre-treatment of any kind of sample: Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents, Before the experiment, each experimental utensil must be checked to 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: The 2 concentrated redissolving solution is mixed with the deionized water at 1:1 (1 mL concentrated redissolving solution + 1 mL deionized water) 0.2 M PB buffer: dissolve 52.0 g Na2HPO412H2O and 8.8 g NaH2PO42H2O in the deionized water to 1 L 5.1 Chicken and Liver Take 20.05 g of the sample, crumble and remove fat, add 8 mL 0.2 M PB buffer, shake properly for 5 min, place in 56oC waterbath for 30 min Centrifuge at above 3000 g at room temperature (20-25oC) for 10 min. Take 50 L supernatant (upper layer), add 450 L of the diluted redissolving solution, mix properly. Take 50 L for analysis. Fold of dilution of the sample: 40 **ELISA procedures** Instructions: Bring all reagents and micro-well strips to the room temperature (20-25oC) before use. Return all reagents to 2-8oC immediately after use. 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. 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. Operating procedures: Take out the kit from the refrigerated environment. Take out all the necessary reagents from the kit and place at the room temperature (20-25oC) for at least 30 min. Note that each liquid reagent must be shaken to mix evenly before use. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2-8oC, not frozen. Solution preparation: dilute 40 mL of the concentrated washing buffer (20 concentrated) with the distilled or deionized water to 800 mL (or just to the required volume) for use.

Numbering: number the micro-wells according to samples and standard solution, each sample and standard solution should be performed in duplicate, record their positions. Add 50 L of the sample or standard solution to separate duplicate wells, and add 50 L enzyme conjugate and then 50 L of the antibody solution into each well. Vortex evenly, seal the microplate with the cover membrane, and incubate at 25oC for 1 h Pour liqiud out of the microwells, add 250 L/well of washing buffer for 10 sec, repeat four to five times, then flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips). Coloration: add 50 L of the substrate A solution and then 50 L of the B solution into each well. Mix gently by shaking the plate manually, and incubate at 25oC for 15 min at dark for coloration, Determination: add 50 L of the stop solution into each well. 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 min). **Interpretation of results** There are two methods to judge the results, the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the content of Gentamicin. 7.1 Qualitative determination The concentration range (ng/mL) can be obtained from the comparison the average OD value of the sample with that of the standard solution. Assuming that the OD value of the sample 1 is 0.50, and that of the sample 2 is 1.0, while those of the standard solutions are as the followings: 1.675 for 0ppb, 1.398 for 0.1 ppb, 1.197 for 0.30 ppb, 0.872 for 0.9 ppb, 0.510 for 2.7 ppb and 0.213 for 8.1 ppb, accordingly the concentration range of the sample 1is 2.7 to 8.1 ppb, and that of the sample 2 is 0.3 to 0.9 ppb. (multiplied by the corresponding dilution fold). 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 100% B0 Bthe average (double wells) OD value of the sample or the standard solution B0the average OD value of the 0ng/mL standard solution. Draw the standard curve with the absorption percentages of the standard solutions and the semilogarithm values of the Gentamicin 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 obtaining the Gentamicin concentration in the sample. Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software) **Precautions** The room temperature below 20oC or the temperature of the reagents and the samples being not returned to the room temperature (20-25oC) will lead to a lower standard OD value. Dryness of the microplate in the washing process will be accompanied by the situations

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	including the non-linear standard curves and the undesirable reproducibility. Mix every reagent and reaction mixture evenly and wash the microplate thoroughly, otherwise there will be the undesirable reproducibility. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin. 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. 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. 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. The optimum reaction temperature is 25oC, and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.
Restrictions:	For Research Use only

Handling

Storage:	4 °C