

## Antibiotics ELISA kits available from ADI:

[http://4adi.com/commerce/catalog/spcategory.jsp?category\\_id=2739](http://4adi.com/commerce/catalog/spcategory.jsp?category_id=2739)

DE-100010	Clenbuterol ELISA kit, 96 tests (For Urine, Serum, Feed, Meat, Liver)
DE-100020	Ractopamine ELISA kit, (For Liver, Urine, Feed), 96 tests
DE-100030	Salbutamal ELISA kit, For Urine, Tissue, Feed, Animal Tissue, Aquatic, Honey, Intestine,, 96 tests
DE-100040	Chloramphenicol ELISA kit, 96 tests (For Animal Tissue, Aquatic, Honey, Intestine, Urine, Egg, Milk, Serum)
DE-100050	Florfenicol ELISA kit (For Animal Tissue, Aquatic, Honey), 96 tests
DE-100060	Nitrofurantoin (AMOZ) ELISA kit (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100070	Nitrofurantoin (AHD) ELISA kit, (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100075	Nitrofurantoin (SEM) ELISA kit (Honey, Fish, Shrimp, Chicken/Liver, Fish/Shrimp), 96 tests
DE-100080	Nitrofurantoin (AOZ) ELISA kit (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100090	Sulfonamides Residues (SAs) ELISA kit, (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine, Milk), 96 tests
DE-100100	Sulfamethazine (SM2) ELISA kit, 96 tests (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine, Milk)
DE-100110	Sulfamethoxydiazine (SMD) ELISA kit, (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine), 96 tests
DE-100120	Quinolones (QNS) ELISA kit (For Pork/Liver, Chicken/Liver, Shrimp, Fish, Serum, Honey), 96 tests
DE-100130	Enrofloxacin ELISA kit (For Pork/Liver, Chicken/Liver, Shrimp, Fish, Serum, Honey), 96 tests
DE-100140	Ampicillin ELISA kit, (For Pork/Liver, Chicken, Duck, Shrimp, Fish, Honey, Milk), 96 tests
DE-100150	Benzyl Penicillin ELISA kit, (For Pork/Liver, Chicken, Duck, Shrimp, Fish, Honey, Milk), 96 tests
DE-100160	Tylosin ELISA kit (For Meat, Liver, Honey, Egg), 96 tests
DE-100170	Trenbolone ELISA kit (For Animal Tissue, Aquatic, Urine), 96 tests
DE-100180	Diazepam ELISA kit (For Tissue, Urine, Feed), 96 tests
DE-100190	Diethylstilbestrol (DES) ELISA kit (Fish, Shrimp, Liver, Meat, Feed, Urine), 96 tests
DE-100200	Gentamicin ELISA kit (Chicken/Liver), 96 tests
DE-100210	Streptomycin ELISA kit, 96 tests (Chicken/Liver, Honey, Milk)
DE-100230	Olaquinox ELISA kit (Tissue) 96 tests
DE-100240	Sulfaquin-oxaline ELISA kit, (For Pork/Liver, Honey/Egg, Serum/Urine, Milk), 96 tests

Instruction Manual No. M-DE-100190

## Diethylstilbestrol ELISA KIT

**Cat. #DE-100190.**

For Qualitative and Quantitative Determination  
of Diethylstilbestrol in shrimp, fish, meat, liver, urine and  
feed



**ALPHA DIAGNOSTIC  
INTERNATIONAL**

**India Contact:**

**Life Technologies (India) Pvt. Ltd.**

306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034  
Ph: +91-11-42208000, 42208111, 42208222

Mobile: +91-9810521400

Fax: +91-11-42208444

Email: [customerservice@atzlabs.com](mailto:customerservice@atzlabs.com)

Web: [www.atzlabs.com](http://www.atzlabs.com)

See Details at the web site or Contact ADI



**Diethylstilbestrol ELISA KIT Cat. #DE-100190**

Kit Components, 96 tests	Cat #
Micro-well coated strip plate (12 strips with 8 removable wells each)	DE-100191
6x standard solution (1 ml each): 0.0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb	DE-100192
Enzyme conjugate (12 mL)	DE-100193
Antibody working solution (7 mL)	DE-100194
Substrate A solution (7 mL)	DE-SSA
Substrate B solution (7 mL)	DE-SSB
Stop solution (7 mL)	DE-ST
20x concentrated washing buffer (40 mL)	DE-WB
20x concentrated redissolving solution (50 mL)	DE-SS20
Instruction Manual	M-DE-100190

**INTRODUCTION**

Diethylstilbestrol (DES) is a synthetic nonsteroidal estrogen, a female drug. It was first synthesized in 1938 and used until 1971 as the purpose of improving the pregnancy outcomes. In 1971 it was found to be dangerous when taken during pregnancy. Its chemical formula is C<sub>18</sub>H<sub>20</sub>O<sub>2</sub> and its molecular weight is 268.35 g/mol. It was first synthesized by Leon Golberg and patent by a German pharmaceutical company Schering. It was synthesized at a very low cost from coal tar.

On September of 1941 United States Food and Drug Administration approved to treat the following conditions; gonorrheal vaginitis, atrophic vaginitis, menopausal symptoms, and postpartum lactation suppression to prevent breast engorgement. However, after penicillin became available DES was no longer an option to treat gonorrheal vaginitis. The period between 1940s until 1980s, FDA approved DES as an estrogen-replacement therapy for estrogen deficiency states such as ovarian dysgenesis, premature ovarian failure and post-oophorectomy. Physicians were prescribing the drug to prevent miscarriage for woman who previously had experienced miscarriage. For that reason DES became very popular between the years of 1953 and 1960. Until 1960 DES was the first choice to treat breast cancer in postmenopausal woman, and it seemed to be more effective than androgens. However in 1977 according to studies the antagonist estrogen receptor, tamoxifen was found to be as effective as DES, but with less side effects. The studies in 1950s showed that DES does not help woman with pregnancy difficulties. Also when DES is given during the first 5 months of pregnancy it can interfere with the growth of the reproductive system in a fetus. As being said, DES was no long prescribed as a drug to help prevent miscarriage. The daughters of women exposed to DES can develop an uncommon cancer of the vagina or cervix which is called adenocarcinoma. The sons of women exposed to DES can have undescended testicles or abnormally small testicles. The women treated with DES might have a slightly increase in

**CALCULATION 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 diethylstilbestrol concentration.

**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 I is 0.310, and that of the sample II is 0.820, the OD value of standard solutions is: 1.510 for 0 ppb, 1.320 for 0.1 ppb, 1.03 for 0.3 ppb, 0.660 for 0.9 ppb, 0.389 for 2.7 ppb, 0.198 for 8.1 ppb, accordingly the concentration range of the sample I is 0.8-1.6 ppb, and that of the sample II is 0.2-0.4 ppb (multiplied by the corresponding dilution fold).

**Quantitative determination**

The mean values of the absorbance values is obtained for 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,

$$\text{Percentage of absorbance value} = \frac{B}{B0} \times 100\%$$

B—the average 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 solution and the semilogarithm values of the Diethylstilbestrol standard solution (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, finally obtaining the Diethylstilbestrol 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).

**Technical specifications**

**Sensitivity:** 0.1 ppb

**Detection limit**

- Shrimp, fish..... 0.2 ppb
- Pork/liver, chicken/liver..... 2 ppb
- Urine.....0.6 ppb
- Feed..... 20 ppb

**Recovery rate**

- Urine.....70±10%
- Feed.....90±10%
- Tissue.....85±10%

**Cross-reaction rate**

- DES..... 100%
- Dienestrol.....38.5%
- Hexestrol.....8.5%
- Ethinylestradiol..... < 0.1%
- Estriol..... < 0.1%

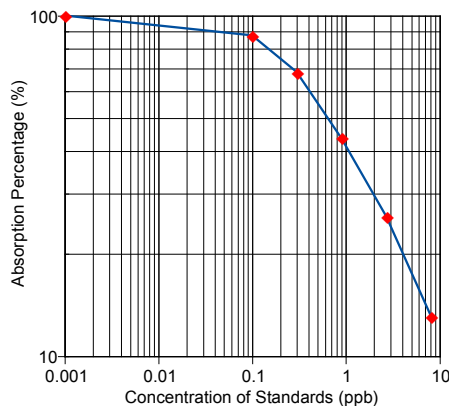
**NOTES:**

1. The room temperature below 20 °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.
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; So continue to next step immediately after washing.
3. Mix evenly, otherwise there will be the undesirable reproducibility.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
5. 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.
6. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colorless color former is light sensitive, and thus they cannot be directly exposed to the light.
7. Discard the coloration solution with any color that indicates the degeneration of this solution. The detecting value of the 0 standard solution of less than 0.5 (A450 nm < 0.5) indicates its degeneration.
8. The optimum reaction temperature is 37 °C, and too high or low temperatures will result in the changes in the detecting sensitivity and OD values.

**Work Sheet of Typical Assay-Diethylstilbestrol**

Wells	Stds/samples	Mean A <sub>450</sub> nm	Absorption Percentage
A1, A2	<b>Standard A</b> 0.0 ppb	1.510	100%
B1, B2	<b>Standard B</b> 0.1 ppb	1.320	87.42%
C1, C2	<b>Standard C</b> 0.3 ppb	1.03	68.21%
D1, D2	<b>Standard D</b> 0.9 ppb	0.660	43.71%
E1, E2	<b>Standard E</b> 2.7ppb	0.389	25.76%
F1, F2	<b>Standard F</b> 8.1 ppb	0.198	13.11%

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical assay Standard Curve (do not use this for calculating sample values)

the probability of having breast cancer comparing to the women that were not exposed to it.

On the other hand, DES has showed good results for dogs and cats. It works as and estrogen which is one of the primary ovarian hormones. DES will activate like the real estrogen and will help the development and maintenance of female sexual organs and secondary sex characteristics.

Alpha Diagnostic Intl's Diethylstilbestrol ELISA kit is a highly sensitive competitive type assay for the measurement of diethylstilbestrol in shrimp, fish, meat, liver, urine and feed

**PRINCIPLE OF THE TEST**

This test kit is based on the competitive enzyme immunoassay for the detection of Diethylstilbestrol (DES) in the feed, urine, liver, meat, shrimp and fish. The conjugate antigen is pre-coated on the micro-well stripes. The Diethylstilbestrol in the sample competes with the conjugate antigen pre-coated on the micro-well stripes, to interact with the antibodies against Diethylstilbestrol. 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 content of Diethylstilbestrol in the sample. This value is compared to the standard curve and the content of the corresponding Diethylstilbestrol is subsequently obtained.

**MATERIALS AND EQUIPMENT REQUIRED**

**Equipments:** micro plate reader, printer, homogenizer, nitrogen-drying device, vortex, centrifuge, measuring pipettes, balance (a sensibility reciprocal of 0.01 g)

**Micropipettors:** single-channel 20 to 100 µL and 100 to 1000 µL, and multi-channel 250 µL.

**Reagents:** NaOH, Acetonitrile (CH<sub>3</sub>CN), Acetone, deionized water, H<sub>3</sub>PO<sub>4</sub> (85%), CHCl<sub>3</sub>.

**PRECAUTIONS AND SAFETY INSTRUCTIONS**

The **Diethylstilbestrol Kit** is for research use only.

**Stop Solution** contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

**MSDS for TMB, sulfuric acid**, if not already on file, can be requested or obtained from the ADI website.

**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. 6 M H<sub>3</sub>PO<sub>4</sub> : dissolve 100 mL H<sub>3</sub>PO<sub>4</sub> in 150 mL deionized water, mix properly.
2. 1 M NaOH : dissolve 4 g NaOH in deionized water to 100 mL.
3. 2 M NaOH : dissolve 8 g NaOH in deionized water to 100 mL.
4. Acetonitrile- Acetone: add 80 mL Acetonitrile and 20 mL Acetone, mix evenly.
5. The 5×concentrated redissolving solution is mixed with deionized water at 1:5 (1 mL concentrated redissolving solution + 4 mL deionized water), used for the treated sample redissolving.

### **Samples preparation**

#### **a) Feed**

1. Weight 2 ± 0.05 g of the homogenized sample, add 8 mL Acetonitrile, shake properly for 10 min, centrifuge at above 3000 r/min at 15 °C for 10 min
2. Take 2 mL supernatant into a new centrifuge tube, blow to dry with nitrogen or air at 60 °C.
3. Add 0.5 mL CHCl<sub>3</sub>, vortex for 20 sec, add 2 mL 1 M NaOH, vortex for 30 sec, centrifuge at above 3000 r/min for 5 min.
4. Take 1 mL supernatant, add 100 µL 6 M H<sub>3</sub>PO<sub>4</sub>, vortex for 5 sec
5. Dilution: Compound feed-- take 50 µL sample, add 950 µL of the diluted redissolving solution. Concentrated /Premixed feed-- take 25 µL sample, add 975 µL of the diluted redissolving solution
6. Take 50 µL for analysis

#### **Fold of dilution of the sample:**

**Compound feed -----100                      Concentrated / Premixed feed -----200**

#### **b) Meat, liver, shrimp, fish**

1. Weigh 2 ± 0.05 g of the homogenized sample, add 6 mL Acetonitrile-Acetone, shake for 10 min, and centrifuge at above 3000 r/min at 15 °C for 10 min.
2. Transfer 3 mL supernatant into a new centrifuge tube, blow to dry with nitrogen or air at 60 °C. Add 0.5 mL CHCl<sub>3</sub>, vortex for 20 sec, add 2 mL 1 M NaOH, vortex for 30 sec, centrifuge at above 3000 r/min for 5 min.
3. Take 1 mL supernatant, add 200 µL 6 M H<sub>3</sub>PO<sub>4</sub>, vortex for 5 sec.
4. Add 3 mL Acetonitrile (CH<sub>3</sub>CN) for extraction, shake properly for 10 min, centrifuge at above 3000 r/min at room temperature (20-25 °C) for 10 min, take the upper layer, blow to dry with nitrogen or air at 60 °C.
5. Dissolve dry residues in 1 mL of the diluted redissolving solution.
6. Dilution: shrimp and fish----directly take 50 µL water phase for detection; Meat and liver----take 50 µL water phase, add 450 µL of the diluted redissolving solution, shake properly.
7. Take 50 µL for analysis.

#### **Fold of dilution of the sample:**

**shrimp and fish----2                                      meat and liver-----20**

#### **C) Urine**

1. Take 2 mL urine into centrifuge tube, centrifuge at above 3000 r/min at room temperature (20-25) for 10 min, stop when it is clear.
2. Transfer 1 mL clear urine into centrifuge tube, add 1 mL 1 M NaOH, shake vigorously for 5 min
3. Add 100 µL 6 M H<sub>3</sub>PO<sub>4</sub>, vortex for 30 sec

4. Add 8 mL CHCl<sub>3</sub> for extraction, shake properly for 10 min, centrifuge at above 3000 r/min at 15 °C for 10 min.
5. Remove the upper layer (water phase), take 4 mL of the lower layer, blow to dry with nitrogen or air at 60 °C.
6. Dissolve dry residues in 3 mL of the diluted redissolving solution.
7. take 50 µL for analysis.

#### **Fold of dilution of the sample:6**

### **STORAGE AND STABILITY**

**Storage:** store at 2 to 8 °C, not frozen.

**Expiration date:** 6 months; date of production is on box.

### **TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).**

#### **Instructions**

1. Bring all reagents and micro-well strips to the room temperature (20-25 °C) before use.
2. Return all reagents to 2-8 °C immediately after use.
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.
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.

#### **Operation procedure**

1. Take out the kit from the refrigerated environment. Take out all the necessary reagents from the kit and place at the room temperature (20-25 °C) for at least 30 min. Note that each liquid reagent must be shaken to mix evenly before use.
2. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2-8 °C, not frozen.
3. Solution preparation: dilute the 20× concentrated washing buffer with the distilled or deionized water to 800 mL (or just to the required volume) for use.
4. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions.
5. Add 50 µL of the sample or standard solution to separate duplicate wells, add 50 µL of the antibody working solution into each well. Seal the microplate with the cover membrane, and incubate at 37 °C for 30 min.
6. Pour liquid out of microwell, add 250 µL/well of washing buffer for 10 sec, repeat four to five times. flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).
7. Add 100 µL enzyme conjugate into every well, seal the microplate with the cover membrane, incubate at 37 °C for 30 min, continue as described in 6.
8. Coloration: add 50 µL substrate A solution and 50 µL B solution into each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane and incubate at 37 °C for 15 min at dark for coloration .
9. Determination: add 50 µL 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).