Antibiotics ELISA kits available from ADI:

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	Nitrofuran (AMOZ) ELISA kit (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100070	Nitrofuran (AHD) ELISA kit, (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100075	Nitrofuran (SEM) ELISA kit (Honey, Fish, Shrimp, Chicken/Liver, Fish/Shrimp), 96 tests
DE-100080	Nitrofuran (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	Olaquindox ELISA kit (Tissue) 96 tests
DE-100240	Sulfaquin-oxaline ELISA kit, (For Pork/Liver, Honey/Egg, Serum/Urine, Milk), 96 tests

See Details at the web site or Contact ADI

Instruction Manual No. M- DE-100180

Diazepam ELISA KIT

Cat. #DE-100180.

For Qualitative and Quantitative Determination of Diazepam in tissue urine, and feed

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LIFE TECH Life Technologies™ Your Molecular & Cell Technology Partner

Diazepam ELISA KIT Cat. #DE-100180

Kit Components, 96 tests	Cat #
Micro-well coated strip plate (12 strips with 8 removable wells each)	DE-100181
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-100182
Enzyme conjugate (7 ml)	DE-100183
Antibody working solution (10 ml)	DE-100184
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
2x concentrated redissolving solution (50 mL)	DE-SS2
Instruction Manual	M- DE-100180

INTRODUCTION

Diazepam is a drug derived from benzodiazepine. Benzodiazepine is an anxiolytic, anticonvulsant, hypnotic, sedative, skeletal muscle relaxant and has amnestic properties. It is a drug used to treat anxiety, insomnia, agitation, seizures, muscle spasms and alcohol withdrawal. It is also used before some medical procedures for example endoscopies or dental work. Its chemical formula is C16H13ClN2O, its molecular weight is 284.7 g/mol. Its physical property is odorless, colorless to light yellow crystalline compound. It is insoluble in water, but soluble in alcohol and very soluble in chloroform. It has a little bitter taste and has a melting point of 131.5 to 134.5°C. It can be absorbed into plastic for that reason it can not be stored in plastic containers.

Diazepam was invented by Stembach of Hoffmann-La roche. It was approved in 1960 and in 1963 it was released as the name of Valium which made Roche a very big industry. From 1969 to 1982 diazepam was the most popular drug sold in the pharmaceutical industry. Its peak was in 1978 with a 2.3 billion tablets sold. Diazepam is commonly prescribed as a medication to relief anxiety for a short period. It is also prescribed to treat neurology conditions such as some types of epilepsy, some forms of paresis, and a rare disorder called stiff-person syndrome.

In human diazepam plays a major role in regulating excitement in the nervous system and regulates the muscle tone. It works by binding to a specific subunit located on the synapse of the neuron in the brain, called gamma-aminobutyric acid (GABA) receptor, GABA is an inhibitory neutransmitter. Diazepam seems to be most active on areas such as limbic system, thalamus and hypothalamus. When GABA is triggered it decreases the activity of the brain, by hyperpolarization of the post-synaptic membrane leading to the opening and closing of ion channels in the cell. The ion channels open and calcium ions which are negatively charged flows into the cell, and potassium ions which are

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 diazepam 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 is0.211, and that of the sample Ⅱ is 0.785, the OD value of standard solutions is: 2.100 for 0 ppb, 1.580 for 0.1 ppb, 1.010 for 0.3 ppb, 0.580 for 0.9 ppb, 0.308 for 2.7 ppb, 0.120 for 8.1 ppb, accordingly the concentration range of the sample Ⅰ is 2.7 to 8.1 ppb, and that of the sample Ⅱ is 0.3 to 0.9 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 Diazepam 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 Diazepam 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
Tissue.....1 ppb
Urine.....1 ppb
Feed.....10 ppb
Recovery rate
Urine.....80±10%
Feed.....75±10%
Tissue (meat, liver).....85±10%
Cross-reaction rate
Diazepam.....100%
Nitrazepam.....7.6%
Oxazepam.....8.8%

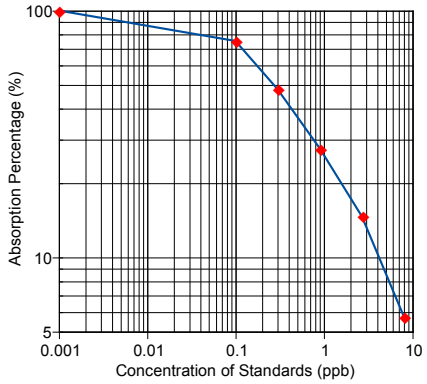
NOTES:

1. The room temperature below 20 ℃ or the temperature of the reagents and the samples being not returned to the room temperature (20-25 ℃) 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. Coloration time is about 15 min, if the color is light, prolong the time of coloration but don't exceed 30 min.
9. The optimum reaction temperature is 25 ℃, and too high or low temperatures will result in the changes in the detecting sensitivity and OD values.

Work Sheet of Typical Assay-Diazepam

Wells	Stds/samples		Mean $A_{450\ nm}$	Absorption Percentage
A1, A2	**Standard A**	0.0 ppb	2.100	100%
B1, B2	**Standard B**	0.1 ppb	1.580	75.24%
C1, C2	**Standard C**	0.3 ppb	1.01	48.10%
D1, D2	**Standard D**	0.9 ppb	0.580	27.62%
E1, E2	**Standard E**	2.7 ppb	0.308	14.67%
F1, F2	**Standard F**	8.1 ppb	0.120	5.71%

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)

positively charged flows out or the cell. There are a few different types of GABA receptors, the specific one that diazepam binds to is called GABAA. GABAA receptors are chloride channels that allow chloride ions to flow inside the cell making the resting potential stable. Once the resting potential is stable it makes very difficult to an excitatory neurotransmitter to depolarize the neuron and produce an action potential.

In animals it is used as a growth promoter and ansiolytic agent. When it is used in overdose in human it can cause many side effects that can continue for up to 4 hours. The symptoms include; drowsiness, mental confusion, hypotension, impaired motor functions and coma.

There are a few way to detect residues of diazepam such as radioimmunoassay, gas chromatography, and ELISA. ELISA is the best way since it is sensitive and low cost technique.

Alpha Diagnostic Intl's Diazepam ELISA kit is a highly sensitive competitive type assay for the measurement of Diazepam in tissue, urine, and feed.

PRINCIPLE OF THE TEST

This test kit is based on the competitive enzyme immunoassay for the detection of Diazepam in the feed, urine, liver and meat. The coupling antigen is pre-coated on the micro-well stripes. The Diazepam in the sample and the coupling antigen pre-coated on the micro-well stripes compete for the anti-Diazepam antibodies. 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 Diazepam in it. This value is compared to the standard curve and the Diazepam concentration is subsequently obtaine

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 200 to 1000 µL, and multi-channel 250 µL.

Reagents: N-hexane, NaOH, Dichloromethane (CH2Cl2), CH3CN.

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Diazepam 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. 1 M NaOH: dissolve 4 g NaOH in 100 mL deionized water.
2. The 2×concentrated redissolving solution is mixed with deionized water at 1:1 (1 mL concentrated redissolving solution + 1 mL deionized water), used for the treated sample redissolving.
3. N-hexane-CH_2Cl_2 solution: $V_{N\text{-}hexane}$: $V_{CH_2Cl_2}$ = 5:3.

Samples preparation

a) Animal tissues (meat, liver)

1. Take the sample, homogenize at 10000 r/min for 1 min,
2. Weigh 2 ± 0.05 g of the homogenized sample, put into 50 mL centrifugal tube, add 5 mL CH_3CN,1 mL 2 M NaOH, shake properly for 10 min, centrifuge at above 4000 r/min at 10 ℃ for 10 min,
3. Take 3 mL supernatant(upper layer) into a new centrifugal tube, add 200 µL 2 M NaOH, 6 mL N-hexane-CH_2Cl_2 solution, shake for 10 min, and centrifuge at above 4000 r/min at 20-25 ℃ for 5 min,
4. Static for 5-10 min, transfer all supernatant into a new centrifugal tube, blow to dry with nitrogen,
5. Dilute residues in 1 mL of the diluted redissolving solution,
6. Take sample solution, dilute at 1:9 (50 µL sample solution + 450 µL diluted redissolving solution),
7. Take 50 µL for further analysis.

Fold of dilution of the sample: 10 Detection limit: 1 ppb

b) Urine

1. Put 1 mL clear sample into 50 mL centrifuge tube, add 4 mL 0.1 M NaOH, shake properly for 2-5 min,
2. Transfer 1 mL liquid into another centrifugal tube, add 10 mL N-hexane, shake for 5 min, and centrifuge at above 4000 r/min at 20-25 ℃ for 5 min,
3. Transfer 5 mL supernatant into a new centrifugal tube, blow to dry with nitrogen,
4. Dilute with 1 mL of the diluted redissolving solution,
5. Take 50 µL for further analysis.

Fold of dilution of the sample: 10 Detection limit: 1ppb

C) Feed

1. Put 1.0 ± 0.05 g feed into 50 mL centrifugal tube, add 6 mL deionized water and 1 mL 1 M NaOH, vortex for 1 min, add 6 mL N-hexane-CH_2Cl_2 solution(5:3), shake properly for 10 min, centrifuge at above 4000 r/min at room temperature for 5 min,
2. Take 3 mL supernatant(upper layer), blow to dry with nitrogen at 50 ℃,
3. Dilute with 1 mL of the diluted redissolving solution .

4. Dilution: at 1:49(10 µL sample + 490 µL the diluted redissolving solution).
5. Take 50 µL for further analysis

Fold of dilution of the sample: 100 Detection limit: 10 ppb

Notice: We recommend the standard 3 as cut off because of some interference.

STORAGE AND STABILITY

Storage: store at 2 to 8 ℃, not frozen.

Expiration date: 12 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 ℃) before use.
2. Return all reagents to 2-8 ℃ 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 ℃) 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 ℃, 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 ℃ for 30 min.
6. Pour liquid out of mirowell, 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 ℃ 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 ℃ 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).