AB302934 – Salbutamol ELISA Kit

For the quantitative measurement of Salbutamol in urine, feed, tissue, liver.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab302934

Storage and Stability:

On receipt entire assay kit should be stored at 2-8°C. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Materials Supplied

Item	Quantity (96T)	Storage Condition
Assay plate	8 x 12 strips	+4°C
Standard	6 x 1 mL	+4°C
HRP-conjugate	1 x 5.5 mL	+4°C
Antibody	1 x 5.5 mL	+4°C
Substrate A	1 x 6 mL	+4°C
Substrate B	1 x 6 mL	+4°C
Stop Solution	1 x 6 mL	+4°C
Redissolving Solution (10X)	1 x 50 mL	+4°C
Wash Buffer 20X	1 x 40 mL	+4°C
Adhesive Strip	4	NA

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm
- An incubator which can provide stable incubation conditions up to 25°C
- Squirt bottle, manifold dispenser, or automated microplate washer
- Centrifuge, Vortex mixer
- Analytical balance, 2 decimal places
- Single-channel micropipette (20-200 µL, 100-1000 µL)
- 300 µL multichannel micropipette
- 100 mL and 500 mL graduated cylinders
- Deionized or distilled water
- Pipettes and pipette tips
- Test tubes for dilution
- NaOH
- Concentrated HCl

PRECAUTIONS The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Δ Note:

- Kindly use graduated containers to prepare the reagent.

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- Bring all reagents to room temperature (20-25°C) before use for 30 min.
- Only the disposable tips can be used for the experiments and the tips must be changed when used for different reagents.
- Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

Reagent Preparation

0.01 M HCl: take 86 µL of **concentrated HCl** and add it to 100 mL of deionized or distilled water.

1 M NaOH take 4 g of **NaOH** into 100 mL of deionized or distilled water.

Redissolving Solution (1X): Take 1 volume of **Redissolving Solution (10x)** and add 9 volumes of distilled or deionized water, mix well. Keep it at 4 °C for one month.

Wash Buffer (1x): If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of **Wash Buffer (20x)** into 380 mL deionized or distilled water to prepare 500 mL of Wash Buffer (1x). Keep it at 4 °C for one month.

Δ Note:

- Abcam is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their experiments.

Standard Preparation

Bring all reagents and samples to room temperature (20~25 °C) before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate. Please follow **Assay Procedure** for obtaining standard curve.

Standard	S1	S2	S3	S4	S5	S6
Concentration (ppb)	0	0.1	0.3	0.9	2.7	8.1

Sample Preparation and Collection

Typical Sample Dynamic Range	
Sample Type	**Recovery rate**
Tissue	80 ± 10 %
Feed	80 ± 20 %
Liver	80 ± 20 %
Urine	90 ± 10 %

Urine Take 1 mL of clear urine into centrifugal tube. If not clear, centrifuge at 4000 rpm for 5 min. Add 4mL **Redissolving solution (1x)**, vortex 30 seconds. Take 50 µL for further analysis. Dilution factor of the samples: 5.

Tissue Weigh 2.00 ± 0.05 g of homogenized sample and put into centrifugal tube. Add 6 mL of **Redissolving solution (1x)** and vortex for 2 min. Centrifuge at 4000 rpm for 10 min. If sample is high in fat, vortex and incubate for 10 min at 85°C, then centrifuge. Take 50 µL of supernatant sample or further analysis. Dilution factor of the samples: 4.

Liver and feed Weigh 1.00 ± 0.05 g of homogenized sample, put into centrifugal tube. Add 4 mL of **0.01 M HCl**, vortex for 2 min. Centrifuge at 4000 rpm for 10 min at room temperature. Adjust to pH 7 using **1M NaOH**. Take 100 µL of supernatant add 400 µL **Redissolving solution (1x),** shake well. Tale 50 µL for further analysis. Dilution factor of the samples: 20.

Assay Procedure

- Bring all reagents and samples to room temperature (20~25°C) before use.
- Centrifuge the samples before the proceeding with assay.
- It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining assay plates and other reagents back into the pouch and seal the Ziploc; store unused wells at 4°C.
3. Add 50 µL of **Standard** or **Sample** per well. Then add 50 µL of **HRP-conjugate** to each well and 50 µL of **Antibody** to each well. Cover the assay plate with a new adhesive strip and mix well, then incubate for 30 min at 25°C.
4. Aspirate each well and wash repeating the process 5 times. Wash by filling each well with 250 µL of Wash Buffer (1x) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 30 seconds. Complete removal of liquid at each step is essential to good performance.
5. Add 50 µL of **Substrate A** and 50 µL of **Substrate B** to each well, mix well. Incubate for 15 minutes at 25°C. Protect from light.
6. Add 50 µL of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well within 10 min, using a microplate reader set to 450 nm (Recommend reading the OD value at the dual wavelength: 450/630 nm).

CALCULATION

The mean values of the absorbance values of the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100 % and the absorbance values are quoted in percentages.

$$Absorbance\ value\ (\%) = \frac{B}{B_0} \times 100\%$$

B – the average absorbance value of sample or standard

B_0 - the average absorbance value of 0 ppb standard

To draw a standard curve: Take the absorbency value of standards as y-axis, logarithmic of the concentration of the Salbutamol (SALB) standards solution (ppb) as x-axis.

The Salbutamol (SALB) concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

Δ Note:

- Discard the substrate with any color that indicates the degeneration of this solution when the absorbance value of standard solution 1 less than 0.5 indicates its degeneration.
- The optimum reaction temperature is 25℃, and the assay temperature conditions lower or higher than that will result in the changes in the absorbance value and detecting sensitivity.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

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