



**(For scientific research use only, not for clinical diagnosis!)**

**Monkey Melatonin (MT)**

**ELISA Kit Instructions for**

**Use Specifications: 48T/96T**

**Please read the instructions carefully before use. If you have any questions,  
please contact us through the following methods: Official hotline: 025-5229-  
8998 Sales department phone: 13914481711 Technical phone: 15950492658  
Contact email: 3224949330@qq.com Company website:  
www.byabscience.cn For specific shelf life, please see the reagents Box  
packaging label. Please use the kit within the shelf life.**

When contacting us, please provide the product number and production date (see box label) so that we can serve you more efficiently.

**Nanjing BYabscience technology Co.,Ltd**

**Website:** [www.byabscience.cn](http://www.byabscience.cn)

**Official hotline:** 025-5229-8998

**Supervision phone number:**



**Kit performance Detection range: 12.5**

**pg/mL–200 pg/mL.**

**Sensitivity:** The lowest detectable dose is less than 1.0 pg/mL.

**Precision:** intra-batch variation coefficient CV% is less than 10%; inter-batch variation coefficient CV% is less than 15%.

**Recovery rate:** The recovery rate is between 85%-115%.

**Specificity:** This kit recognizes natural and recombinant monkey melatonin (MT) and has no crossover with structural analogs.

**Stability:** Stored at 2°C–8°C, validity period is 6 months.

**Purpose:** Used to detect the concentration of monkey melatonin (MT) in samples such as serum, plasma, cell culture supernatant and tissue. **Shelf life:** Stored at 2°C–8°C, valid for 6 months.

## **Experimental principle**

The kit uses enzyme-linked immunoassay method. Biotin-labeled MT is used, and the purified anti-MT antibody is coated on the microwell plate. In the competitive inhibition reaction, a certain amount of solid-phase antibody is used to inhibit the competitive reaction with biotin-labeled MT and non-labeled antigen (calibrator or specimen). The antibody The amount of binding to biotin-labeled MT is inhibited by the amount of non-labeled antigen. The greater the amount of non-labeled antigen, the less the antibody will bind to biotin-labeled MT, and vice versa. After the reaction is balanced, a solid-phase antibody-biotinylation is formed. MT, and then add enzyme-labeled avidin to form a solid-phase antibody-biotinylated MT-enzyme label-avidin complex. After adding substrate for color development, use a microplate reader to measure the absorbance (OD value) at a wavelength of 450 nm. As the MT concentration increases, the OD value gradually decreases with a good linear

relationship. This kit has the characteristics of high sensitivity, strong specificity, good repeatability, simple and rapid operation, and has reliable detection performance for the reduction or increase of MT in serum.

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**Kit components and storage: Store unopened kits at 2-8**

**degrees Celsius. Do not use expired kits.**

Components	48-well configuration	96-well configuration	Store after opening
Pre-coated enzyme plate	48T	96T	<b>2-8°C 14 days</b>
Standard product	0.3mL*6 tubes	0.3mL*6 tubes	<b>2-8°C 14 days</b>
sample diluent	3ml	6ml	2-8°C 180 days
biotinylated antigen	3ml	6ml	<b>2-8°C 14 days</b>
HRP labeled avidin	3ml	6ml	2-8°C 180 days
Chromogenic substrate A	3ml	6ml	2-8°C 180 days
Chromogenic substrate B	3ml	6ml	2-8°C 180 days
stop solution	3ml	6ml	2-8°C 180 days
<b>20×Lotion</b>	15ml	25ml	2-8°C 180 days
sealing film	2 sheets	2 sheets	
manual	1 serving	1 serving	
Ziplock bag	1	1	

The concentrations of calibrators are: 200, 100, 50, 25, 12.5, 0 pg/mL.

Note: 1: Before use, please check whether the label and quantity of

the reagents in the kit are consistent with the table.

2: If the components of the kit need to be used again, please ensure that they have

not been contaminated since the last use. 3: If the enzyme plate is not used up in a

single time, remember to seal it and store it at 2-8°C.

**Prepare your own test equipment required for the test (not provided, but can assist in**

1) Microplate reader capable of detecting absorbance at 450 nm 2) Pipette,

pipette tip, and sample addition tank 3) 37°C incubator or water bath 4) Test

tubes, centrifuge tubes, measuring cylinders, etc. for preparing reagents 5)

Distilled water or deionized water Ionized water

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6) Vortex oscillator and microplate oscillator.

Notes: 1) For scientific research use only,  
not for clinical diagnosis.

2) Use within the validity period marked on the kit. Expired products must not be used.

3) Do not mix with kits or components from other manufacturers. Use the sample diluent provided with the kit.

4) If the sample value is higher than the highest standard concentration value, please dilute the sample appropriately and then re-measure.

5) Human anti-mouse and other heterophilic antibodies present in the sample to be tested will interfere with the test results. Please eliminate this factor before testing.

6) The test results obtained by other methods are not directly comparable to the test results of this kit.

7) Please wear a lab coat and latex gloves for protection during the test. Especially when testing blood or other body fluid samples, please follow the national biological laboratory safety protection regulations.

8) Carry out incubation strictly according to the specified time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Store reagents refrigerated immediately after use.

9) Improper plate washing can lead to inaccurate results. Make sure to absorb as much liquid as possible from the wells before adding substrate. Do not allow the microwells to dry out during incubation.

10) Eliminate residual liquid and fingerprints on the bottom of the plate, otherwise it will affect the OD value.

11) The substrate chromogenic solution should be colorless or very light in color.

12) Avoid cross-contamination of reagents and specimens to avoid erroneous results.

- 13) Avoid direct exposure to strong light during storage and incubation.
- 14) The microplate reader used for detection needs to be equipped with a filter capable of detecting a wavelength of  $450\pm 10\text{nm}$ , and the optical density range is between 0-3.5. It is recommended to preheat 15 minutes in advance before use.
- 15) The EP tubes and tips used in the test are single-use and are strictly prohibited from mixing.

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## Sample preparation and storage

The following lists only general guidelines for sample collection and preservation. During the collection and storage of all samples, sodium azide must not be used as a preservative. If the sample is not analyzed immediately, it should be aliquoted and stored frozen, and repeated freezing and thawing should be avoided.

**Cell culture supernatant - centrifuge to remove precipitate, analyze immediately or aliquot and store frozen at -20°C.**

**Serum - Collect blood in a clean test tube, coagulate at room temperature for 30 minutes, centrifuge at 2000×g for 20 minutes, and collect serum. Analyze immediately or aliquot and store frozen at -20°C.**

**Plasma—anticoagulate with heparin, citrate, or EDTA, and centrifuge at 2000×g for 20 minutes at 2-8°C within 30 minutes of blood draw. To eliminate the influence of platelets, it is recommended to further centrifuge at 10,000 × g for 10 minutes at 2-8°C. Analyze immediately or aliquot and store frozen at -20°C.**

**Cell lysis buffer - For adherent cells, remove the culture medium and wash with PBS, normal saline or serum-free culture medium. Add an appropriate amount of lysis solution and pipet several times with a gun to fully contact the lysate and cells. Typically after 10 seconds, cells are lysed. For suspended cells, collect the cells by centrifugation and wash them with PBS, physiological saline or serum-free culture medium. Add an appropriate amount of lysis solution, blow the cells with a gun, and flick them with your fingers to fully lyse the cells. After full lysis, centrifuge at 10000-14000×g for 3-5 minutes and take the supernatant. Analyze immediately or aliquot and store frozen at -20°C.**

**Tissue homogenate - rinse the tissue with pre-cooled PBS (0.01M, pH=7.4) to remove residual blood (lysed red blood cells in the homogenate will affect the measurement results), weigh and cut the tissue into pieces. Mix the minced tissue with the corresponding volume of PBS (generally**

at a weight-to-volume ratio of 1:9, for example, 1g of tissue sample corresponds to 9mL of PBS. The specific volume can be adjusted appropriately according to experimental needs and recorded. It is recommended to add Protease inhibitor) was added to a glass homogenizer and ground thoroughly on ice. In order to further lyse tissue cells, the homogenate can be sonicated or repeatedly frozen and thawed. Finally, centrifuge the homogenate at  $5000 \times g$  for 5 to 10 minutes, and take the supernatant for detection.

**Urine - Collect in sterile tubes and centrifuge at  $2000 \times g$  for 20 minutes. Carefully collect the supernatant. If a precipitate forms, centrifuge again.**

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**Reagent preparation 1. Before use, all components must be rewarmed**

**for at least 60 minutes to ensure sufficient rewarming to room temperature.**

2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating in a water bath will completely dissolve the crystals. Concentrated detergent and distilled water, dilute 1:20, that is, 1 part of concentrated detergent, add 19 parts of distilled water.

**Operating procedures: Return all reagents and components to room temperature first. It is recommended to do duplicate holes for standards, quality control materials and samples.**

1. Prepare the working solution of various components of the kit according to the method described in the previous instructions.
2. Take out the required slats from the aluminum foil bag, seal the remaining slats in a ziplock bag and return it to the refrigerator.
3. Take out the pre-coated plate from the sealed bag and set up a blank control well without adding any liquid; set up 2 wells for each calibrator and add 50  $\mu$ l of the corresponding calibrator into each well; add the serum to be tested directly to each of the remaining detection holes. Or 50 $\mu$ l of quality control product.
4. Add 50  $\mu$ l of biotinylated antigen to all wells except the blank well, mix well, attach sealing film, and incubate at 37°C for 60 minutes.
5. Manual plate washing: discard the liquid in the wells, fill each well with washing solution, let stand for 10 seconds and spin dry, repeat 3 times and pat dry. Wash the plate with a plate washer: select the washing program 3 times and pat dry after washing the plate.

**(Tip: In order to obtain ideal experimental results, the residual liquid must be completely removed. After washing the plate, please proceed to the next step immediately and do not let the microplate dry.) 6. Add 50 µl of enzyme-labeled avidin to each well (blank (Excluding control wells), mix well, affix sealing film, and incubate at 37°C for 30 minutes.**

7. Manual plate washing: discard the liquid in the wells, fill each well with washing solution, let stand for 10 seconds and spin dry, repeat 3 times and pat dry. Wash the plate with a plate washer: select the washing program 3 times and pat dry after washing the plate.

8、每孔加显色剂 A 50µl，显色剂 B 50µl，振荡混匀后，置 37°C避光显色 15 分钟，每孔加终止液 50µl。

9、用酶标仪读数，取波长 450nm，先用空白对照孔调零点，然后测定各孔光密度值（OD 值）。

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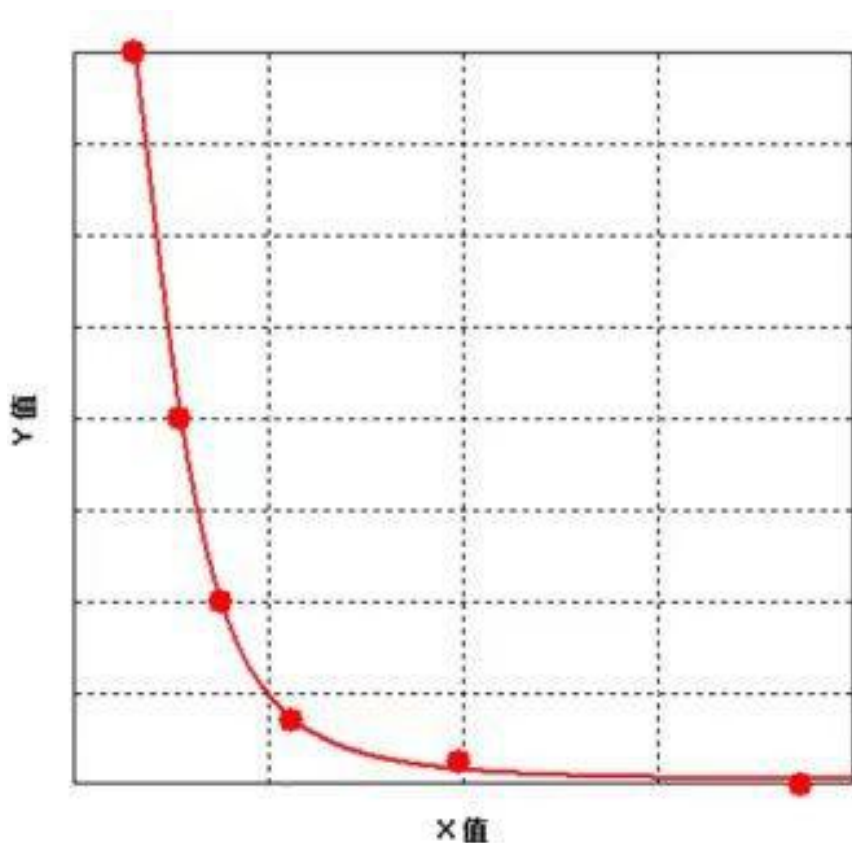
官方热线: 025-5229-8998

监督电话: 15950492658



## 结果计算

9、以标准品浓度做为横坐标，对应的吸光度（OD 值）作为纵坐标，利用计算机软件，采用四参数 Logistic 曲线拟合（4-pl），创建标准曲线方程，通过样本的吸光度（OD 值），利用方程计算样品的浓度值。【用 ELISA Calc 软件计算】10、如果样品被稀释，通过上述方法测得的浓度值，要乘以稀释倍数，才是样品的最终浓度。注意：实验者需根据自己的实验建立标准曲线。每次检测，每块酶标板都必须设立标准曲线。以下曲线仅供参考！



（标曲示意图，仅供参考）

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[问题分析] 若实验效果不好，请及时对显色结果拍照，保存实验数据，保留所用板条及未使用试剂，然后联系我公司技术支持为您解决问题。同时您也可以参考以下资料：[问题解答]

问题描述	可能原因	相应对策
标准曲线梯度差	吸液或加液不准	检查移液器及吸头
	平衡时间太短	保证充足的平衡时间
	洗涤不完全	保证洗涤时间和洗涤次数及每孔的加液量
显色很弱或无色	孵育时间太短	保证充足的孵育时间
	实验温度不正确	使用推荐的实验温度
	试剂体积不够或漏加	检查吸液及加液过程，保证所有试剂按顺序足量添加
	稀释不正确	
读数数值低	酶标仪设置不正确	在酶标仪上检查波长及滤光片设置
		提前打开酶标仪预热
变异系数大	加液不正确	检查加液情况
背景值高	检测抗体的工作浓度过高	使用推荐的稀释倍数
	酶标板洗涤不完全	保证每步清洗完全；如果用自动洗板机，请检查所有的出口是否有堵塞；是否使用试剂盒配备的洗涤液
	洗液有污染	配制新鲜的洗液
灵敏度低	Improper storage of ELISA kits	Store relevant reagents according to
	Not terminated before reading	Stop solution should be added to

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