Chicken Infectious Laryngotracheitis antibody(ILT-Ab) ELISA Kit

- **For research use only.**
- **Our Chicken Infectious Laryngotracheitis antibody(ILT-Ab) ELISA kit is to assay ILT-Ab levels in Chicken serum, plasma, culture media or any biological fluid.**
- **Expiration date：six months .**
- **Storage： 2-8℃.**

Principle

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antigen specific to ILT-Ab. Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antigen. Then a Horseradish Peroxidase (HRP)-conjugated antigen specific forILT added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain ILT-Ab and HRP conjugated ILT will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The presence of ILT-Ab is determined by comparing with the CUTOFF value.

Materials provided with the kit

	Materials provided with the kit	48 determinations	Storage
1	User manual	1	R.T.
2	Closure plate membrane	2	R.T.
3	Sealed bags	1	R.T.
4	Microelisa stripplate	1	2-8℃
5	Negative control	0.5ml×1 bottle	2-8℃
6	Positive control	0.5ml×1 bottle	2-8℃
7	HRP-Conjugate reagent	3ml×1 bottle	2-8℃
8	Sample diluent	3ml×1 bottle	2-8℃
9	Chromogen Solution A	3ml×1 bottle	2-8℃
10	Chromogen Solution B	3ml×1 bottle	2-8℃
11	Stop Solution	3ml×1 bottle	2-8℃
12	wash solution	20ml (20X)×1bottle	2-8℃

Sample preparation

1. **Serum preparation**

After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifugated again.

2. **Plasma preparation**

Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubated at

room temperature for 10-20 minutes, tubes are centrifugated for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during reservation, the sample should be centrifugated again.

3. **Urine samples**

Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine sample.

4. **Cell samples**

If you want to detect the secretions of cells, collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1X106/ml with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again.

5. **Tissue samples**

Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80℃ for future use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be operated at 4℃. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.

Notes:

1. Sample extraction and ELISA assay should be performed as soon as possible after sample collection. The samples should be extracted according to the relevant literature. If ELISA assay can not be performed immediately, samples can be stored at -20 ℃ .Repeated freeze-thaw cycles should be avoided.

2. Our kits can not be used for samples with NaN3 which can inhibit the activity of HRP.

Assay procedure

Procedure

1. In the Microelisa stripplate, leave two wells as negative control, two wells as positive control and one well empty as as blank control. Number: the sequential number, corresponding sample of the microporous hole 2 per board should set negative control and positive control 2 holes, ck 1 hole (ck hole without samples and HRP-Conjugate reagent, the rest of the same step operation)
2. Adding samples: Negative and positive control in a volume of 50μl are added to the negative and positive control wells respectively. In sample wells, 40μl Sample dilution buffer and 10μl sample are added. Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. Incubation: incubate 30 min at 37℃ after sealed with Closure plate membrane.
4. Dilution: dilute the concentrated washing buffer with distilled water (20 times for 48T).
5. Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
6. Add 50 μl HRP-Conjugate reagent to each well except the blank control well.
7. Incubation as described in Step 3.
8. Washing as described in Step 5.
9. Coloring: Add 50 μl Chromogen Solution A and 50 μl Chromogen Solution B to each well, mix with gently shaking and incubate at 37℃ for 15 minutes. Please avoid light during coloring.
10. Termination: add 50 μl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

Determine the result

Test effectiveness: the average value of positive control ≥1.00; the average value of negative control ≤0.10.

The critical value (CUT OFF) calculation: critical value = the average value of negative control + 0.15

Negative judgement: if the OD value< CUT OFF, the sample is Chicken ILT-Ab negative.

Positive judgement: if the OD value ≥CUT OFF, the sample is Chicken ILT-Ab positive.

Notes

2. Store the kit at 4° C upon receipt.The kit should be equilibrat4ed to room temperature before the assay. Remove any unneeded strips from ILT antigen -Coated plate, reseal them in zip-lock foil and keep at 4° C.
3. Precipitates may appear in concentrated washing buffer. Please heat the buffer to dissolve all the precipitates, which will not affect the results.
4. In order to avoid cross-contamination, Closure plate membranes are for one-time use only.
5. Please keep Substrate away from light.
6. All the operation should be accordance with the manufacturer's instructions strictly. The results determined by the Microelisa stripplate Reader.
7. All the samples, washing buffer and wastes should be treated as infectious agents.
8. Reagents from different lots should not be mixed.

Storage and validity

1. Storage： 2-8℃.
2. Duration: 6 months

鸡传染性喉气管炎抗体(ILT-Ab)酶联免疫分析(ELISA)试剂盒使用说明书

- 本试剂盒仅供科研使用。
- 本试剂盒用于体外定性检测鸡血清、血浆、组织、细胞上清及相关液体样本中鸡传染性喉气管炎抗体(ILT-Ab)水平。
- 有效期：6个月
- 保存条件：**2-8**℃

实验原理：

本试剂盒采用双抗原夹心酶联免疫法（ELISA）测定标本中鸡传染性喉气管炎抗体(ILT-Ab)。用纯化的鸡传染性喉气管炎抗原包被微孔板，制成固相抗原，可与样品中鸡传染性喉气管炎抗体(ILT-Ab)相结合，经洗涤除去未结合的抗体和其他成分后再与 HRP 标记的鸡传染性喉气管炎抗原结合，形成抗原-抗体-酶标抗原复合物，经过彻底洗涤后加底物 TMB 显色。TMB 在 HRP 酶的催化下转化成蓝色，并在酸的作用下转化成最终的黄色。用酶标仪在 450nm 波长下测定吸光度（OD 值），与 CUTOFF 值相比较，从而判定标本中鸡传染性喉气管炎抗体(ILT-Ab)的存在与否。

试剂盒组成：

试剂盒组成	48 孔配置	96 孔配置	保存
说明书	1 份	1 份	R.T.
封板膜	2 片（48）	2 片（96）	R.T.
密封袋	1 个	1 个	R.T.
酶标包被板	1×48	1×96	2-8℃保存
阴性对照	0.5ml×1 瓶	0.5ml×1 瓶	2-8℃保存
阳性对照	0.5ml×1 瓶	0.5ml×1 瓶	2-8℃保存
酶标试剂	3 ml×1 瓶	6 ml×1 瓶	2-8℃保存
样品稀释液	3 ml×1 瓶	6 ml×1 瓶	2-8℃保存
显色剂 A 液	3 ml×1 瓶	6 ml×1 瓶	2-8℃保存
显色剂 B 液	3 ml×1 瓶	6 ml×1 瓶	2-8℃保存
终止液	3ml×1 瓶	6ml×1 瓶	2-8℃保存
浓缩洗涤液	（20ml×20 倍）×1 瓶	（20ml×30 倍）×1 瓶	2-8℃保存

样本处理及要求：

1. 血清：室温血液自然凝固 10-20 分钟，离心 20 分钟左右（2000-3000 转/分）。仔细收集上清，保存过程中如出现沉淀，应再次离心。
2. 血浆：应根据标本的要求选择 EDTA 或柠檬酸钠作为抗凝剂，混合 10-20 分钟后，离心 20 分钟左右（2000-3000 转/分）。仔细收集上清，保存过程中如有沉淀形成，应该再次离心。
3. 尿液：用无菌管收集，离心 20 分钟左右（2000-3000 转/分）。仔细收集上清，保存过程中如有沉淀形成，应再次离心。胸腹水、脑脊液参照实行。
4. 细胞培养上清：检测分泌性的成份时，用无菌管收集。离心 20 分钟左右（2000-3000 转/分）。仔细收集上清。检测细胞内的成份时，用 PBS（PH7.2-7.4）稀释细胞悬液，细胞浓度达到 100 万/ml 左右。通过反复冻融，以使细胞破坏并放出细胞内成份。离心 20 分钟左右（2000-3000 转/分）。仔细收集上清。保存过程中如有沉淀形成，应再次离心。
5. 组织标本：切割标本后，称取重量。加入一定量的 PBS，PH7.4。用液氮迅速冷冻保存备用。标本融化后仍然保持 2-8℃的温度。加入一定量的 PBS（PH7.4），用手工或匀浆器将标本匀浆充分。离心 20 分钟左右（2000-3000 转/分）。仔细收集上清。分装后一份待检测，其余冷冻备用。

6. 标本采集后尽早进行提取，提取按相关文献进行，提取后应尽快进行实验。若不能马上进行试验，可将标本放于-20℃保存，但应避免反复冻融.
7. 不能检测含 NaN3 的样品，因 NaN3 抑制辣根过氧化物酶的（HRP）活性。

操作步骤：

1. 编号：将样品对应微孔按序编号，每板应设阴性对照 2 孔、阳性对照 2 孔、空白对照 1 孔（空白对照孔不加样品及酶标试剂，其余各步操作相同）
2. 加样：分别在阴、阳性对照孔中加入阴性对照、阳性对照 50μl。然后在待测样品孔先加样品稀释液 40μl，然后再加待测样品 10μl。加样将样品加于酶标板孔底部，尽量不触及孔壁，轻轻晃动混匀，
3. 温育：用封板膜封板后置 37℃温育 30 分钟。
4. 配液：将 30（48T 的 20 倍）倍浓缩洗涤液加蒸馏水至 600ml 后备用
5. 洗涤：小心揭掉封板膜，弃去液体，甩干，每孔加满洗涤液，静置 30 秒后弃去，如此重复 5 次，拍干。
6. 加酶：每孔加入酶标试剂 50μl，空白孔除外。
7. 温育：操作同 3。
8. 洗涤：操作同 5。
9. 显色：每孔先加入显色剂 A 50μl，再加入显色剂 B 50μl，轻轻震荡混匀，37℃避光显色 15 分钟
10. 终止：每孔加终止液 50μl，终止反应（此时蓝色立转黄色）。
11. 测定：以空白空调零，450nm 波长依序测量各孔的吸光度（OD 值）。 测定应在加终止液后 15 分钟以内进行。

结果判定：

试验有效性：阳性对照孔平均值≥1.00; 阴性对照平均值≤0.10

临界值（CUT OFF）计算：临界值=阴性对照孔平均值+0.15

阴性判定：样品 OD 值< 临界值（CUT OFF）者为鸡传染性喉气管炎抗体(ILT-Ab)阴性

阳性判定：样品 OD 值≥ 临界值（CUT OFF）者为鸡传染性喉气管炎抗体(ILT-Ab)阳性

注意事项

1．操作严格按照说明书进行，本试剂不同批号组分不得混用。
2．试剂盒从冷藏环境中取出应在室温平衡 15-30 分钟后方可使用，酶标包被板开封后如未用完，板条应装入密封袋中保存。
3．浓洗涤液可能会有结晶析出，稀释时可在水浴中加温助溶，洗涤时不影响结果。
4．封板膜只限一次性使用，以避免交叉污染。
5．底物请避光保存。
6．试验结果判定必须以酶标仪读数为准，使用双波长检测时，参考波长为 630nm
7．所有样品，洗涤液和各种废弃物都应按传染物处理。终止液为 2M 的硫酸，使用时必须注意安全。

保存条件及有效期

1．试剂盒保存:；2-8℃。
2．有效期：6 个月