犬钩端螺旋体 IgG 抗体(Lep-IgG)酶联免疫分析（ELISA）

试剂盒使用说明书　　AE91826Ca-Q

本试剂仅供研究使用　　目的：本试剂盒用于检测犬血清，血浆及相关液体样本中钩端螺旋体 IgG 抗体(Lep-IgG)水平。

实验原理：

本试剂盒采用双抗原夹心酶联免疫法（ELISA）测定标本中犬钩端螺旋体 IgG 抗体(Lep-IgG)。用纯化的抗原包被微孔板，制成固相抗原，可与样品中钩端螺旋体 IgG 抗体(Lep-IgG)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，从而判定标本中犬钩端螺旋体 IgG 抗体(Lep-IgG)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	
封板膜	2 片（48）	2 片（96）	
密封袋	1 个	1 个	
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：以空白孔调零，450nm 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≤0.20

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）者为犬钩端螺旋体 IgG 抗体(Lep-IgG)阴性

阳性判定：样品 OD 值≥ 临界值（CUT OFF）者为犬钩端螺旋体 IgG 抗体(Lep-IgG)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

Canine Lep-IgG

FOR RESEARCH USE ONLY

Drug Names

Generic Name：**Canine Lep-IgG ELISA Kit.**

Purpose

This kit allows for the determination of Lep-IgG concentrations in Canine serum, and other biological fluids.

Principle of the assay

The kit assay Lep-IgG level in the sample， use Purified antigen to coat microtiter plate wells, make solid-phase antigen, then add Lep-IgG to wells, Combined With Lep-IgG, after washing and removing non-combinative antibody and other components ,then Combined antigen which with HRP labeled become antigen – antibody - enzyme- antigen complex, after washing Completely, Add TMB substrate solution,, TMB substrate becomes blue color At HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. Compared with the CUTOFF value, according to this to judge Lep-IgG exist in the sample or not.

Materials provided with the kit

Materials provided with the kit	48determinations	96 determinations	Storage
User manual	1	1	
Closure plate membrane	2	2	
Sealed bags	1	1	
Microelisa stripplate	1	1	2-8℃
Negative control	0.5ml×1 bottle	0.5ml×1 bottle	2-8℃

Positive control	0.5ml×1 bottle	0.5ml×1 bottle	2-8℃
HRP-Conjugate reagent	3ml×1 bottle	6ml×1 bottle	2-8℃
Sample diluent	3ml×1 bottle	6ml×1 bottle	2-8℃
Chromogen Solution A	3ml×1 bottle	6ml×1 bottle	2-8℃
Chromogen Solution B	3ml×1 bottle	6ml×1 bottle	2-8℃
Stop Solution	3ml×1 bottle	6ml×1 bottle	2-8℃
wash solution	（20ml×20 fold）×1bottle	（20ml×30 fold）×1bottle	2-8℃

Specimen requirements

1. **serum-** coagulation at room temperature 10-20 mins，centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.
2. **plasma-**use suited EDTA or citrate plasma as an anticoagulant,mix 10-20 mins ,centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.
3. **Urine**-collect sue a sterile container, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again. The Operation of Hydrothorax and cerebrospinal fluid Reference to it.
4. **cell culture supernatant**-detect secretory components, collect sue a sterile container, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant,detect the composition of cells, Dilut cell suspension with PBS（PH7.2-7.4）, Cell concentration reached 1 million / ml, repeated freeze-thaw cycles, damage cells and release of intracellular components, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.
5. **Tissue samples**- After cutting samples, check the weight,add PBS （PH7.2-7.4）, Rapidly frozen with liquid nitrogen, maintain samples at 2-8℃ after melting,add PBS（PH7.4）, Homogenized by hand or Grinders,

centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant.

6. extract as soon as possible after Specimen collection,and according to the relevant literature, and should be experiment as soon as possible after the extraction. If it can't, specimen can be kept in -20 ℃ to preserve, Avoid repeated freeze-thaw cycles.
7. Can't detect the sample which contain NaN3, because NaN3 inhibits HRP active.

Assay procedure

1.Number: to sample correspond microtitration well and Number Sequence, each plate should be set feminine comparison 2 wells, masculine comparison 2 wells, blank comparison 1 well(don't add sample and HRP-Conjugate reagent to blank comparison well, other each step the operation are same).

2.add sample：separately add Positive control and Negative control 50μl to the Positive and Negative well . add Sample dilution 40μl to testing sample well, then add testing sample 10μl. add sample to the bottom of ELISA plates coated well , don't touch the well wall as far as possible, and Gently mix.

3.Incubate: After closing plate with Closure plate membrane ,incubate for 30 min at 37℃.

4.Configurate liquid: 30-fold (or 20-fold)wash solution diluted 30-fold (or 20-fold) with distilled water until 600ml,and reserve.

5.washing：Uncover Closure plate membrane, discard Liquid, dry by swing, add washing buffer to every well, still for 30s then drain, repeat 5 times, dry by pat.

6.add enzyme：Add HRP-Conjugate reagent 50μlto each well, except the blank well.

7.incubate：Operation with 3.

8.washing：Operation with 5.

9.color：Add Chromogen Solution A 50ul and Chromogen Solution B to each well, evade the light preservation for 15 min at 37℃

10.Stop the reaction：Add Stop Solution50μl to each well, Stop the reaction(the blue color change to yellow color).

11. assay：take blank well as zero , Read absorbance at 450nm after Adding Stop Solution and within 15min.

Determine the result

Test validity: the average of Positive control well≥1.00; the average of Negative control well ≤0.20.

Calculate Critical(CUT OFF) : Critical= the average of Negative control well + 0.15.

Negative control: sample OD< Calculate Critical(CUT OFF) is Lep-IgG Negative control.

Positive control: ample OD≥ Calculate Critical(CUT OFF) is Lep-IgG Positive control.

Important notes

1.Please according to use instruction strictly, Do not mix reagents with those from other lots.

2.The kit takes out from the refrigeration environment should be balanced 15-30 minutes in the room temperature then use, ELISA plates coated if has not use up after opened, the plate should be stored in Sealed bag.

3.washing buffer will Crystallization separation, it can be heated the water helps dissolve when dilute . Washing does not affect the result.

4.Closure plate membrane only limits the disposable use, in order to avoid

the overlapping pollution

5.The substrate please evade the light preservation.

6.The test result determination must take the microtiter plate reader as a standard, when use dual-wavelength to assay, Reference wavelength is 630nm.

7.All samples, washing buffer and each kind of reject should according to infective material process. Stopp Solution is 2M sulphuric acid. You must pay attention to safe when use .

Storage and validity

1. Storage： 2-8℃.
2. validity： six months.