人激活素 A（ATA）

产品编号：HEA002

Elisa 试剂盒使用说明书

用途

本试剂盒仅供科研使用，定量检测细胞液、体液、组织、血清、血浆等标本中人激活素 A（ATA）的含量。

原理:

采用双抗体夹心法测定人激活素 A（ATA）水平。用纯化的人激活素 A（ATA）抗体包被微孔板，制成固相载体，实验时依次在微孔板中加入样本及标准品，并加入 HRP 标记的 ATA 抗体，形成抗体-抗原-酶标抗体复合物，反复洗涤后加底物 TMB 显色。TMB 在过氧化物酶的催化下转化为蓝色，再用硫酸终止反应，转变成黄色。颜色的深浅和样品中的 ATA 含量呈正相关。采用酶标仪在 450nm 波长测定吸光度（OD 值），根据标准曲线，计算测试样品中 ATA 浓度。

试剂盒组成

序号	名称	规格
1	酶标包被板	96 孔*1 可拆卸板
2	酶结合物	10ml*1 瓶
3	标准品	1ml*6 瓶
4	缓冲液	6ml*1 瓶
5	显色剂 A 液	6ml*1 瓶
6	显色剂 B 液	6ml*1 瓶
7	终止液	6ml*1 瓶
8	浓缩洗涤液（*100）	50ml*1 瓶
9	封板膜	1 张
10	使用说明书	1 份
备注	1.标准品 1～6 的浓度分别为：0、50、100、250、500、1000pg/ml 2.缓冲液仅适用于细胞液、体液以及组织匀浆样品的检测。	

实验材料与试剂配制:

1. 仪器与材料：酶标仪（使用前预热30分钟），微量加液器、吸头、蒸馏水或去离子水，滤纸。
2. 缓冲液使用：加5ul 的缓冲液于50ul 的样品中，如果样品量不够或者不确定，缓冲液和样品的混合比例不要小于1:10即可。混匀，静置1小时备用（如果标本是血清或者血浆，此步骤忽略）。
3. 洗液的配制：按1:100的比例配制洗液备用。

样品收集、处理及保存

1. 细胞培养上清：适用于检测体外培养的细胞分泌性成份。用无菌管收集细胞上清液，以1000×g离心15分钟，收集上清。
2. 细胞：用PBS反复洗涤细胞3次，调整细胞浓度达到10^4-10^6/ml 左右，通过反复冻融，使细胞破坏并放出细胞内成份，或者细胞超声粉碎，离心取上清液检测。
3. 血清：在室温下，血液自然凝固，以1000×g离心15分钟，取上清待测。
4. 血浆：应根据标本的要求选择EDTA 或柠檬酸钠作为抗凝剂，混合后静置10-20 分钟后，以1000×g离心15分钟，收集上清。
5. 体液：包括胸腹水、脑脊液，分泌物等。使用不含热原和内毒素的离心管收集，以1000×g离心15分钟，收集上清。
6. 组织标本：切取组织标本，称取重量0.5mg，加入500ul 的PBS，用手工或匀浆器，或超声破碎仪将标本匀浆，以2000-3000 rmp离心20 分钟，收集上清进行检测。
7. 样品中不能含有NaN3，因NaN3 抑制辣根过氧化物酶的（HRP）活性。
8. 保存：如果样品不能立即检测，应将其分装，-70 ℃保存，避免反复冷冻。保存过程中如出现沉淀，应再次离心。血液标本尽可能的不要使用溶血或高血脂血。如果血清中含大量颗粒，检测前先离心或过滤。不要在37℃或更高的温度加热解冻。应在室温下解冻并确保样品均匀地充分解冻。

操作步骤：

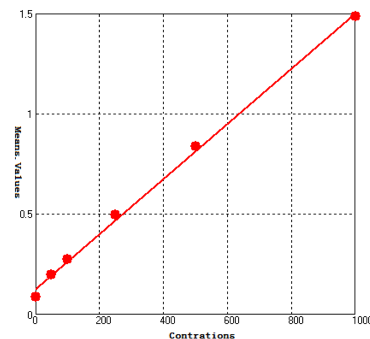
1. 取出试剂盒，室温（20-25℃）放置 30 分钟。
2. 分组：取出 96 孔板，根据待测样品数量加上标准品的数量决定所需的板条数，把剩余的板条继续冷藏处理。分别设标准品组（6 个浓度）、空白孔、待测样品组。

   注：为减少实验误差，保证准确性，建议设置复孔。
3. 加样：依照标准品的顺序分别加入 50ul 的标准品溶液于空白微孔中；空白对照孔加入 50ul 的蒸馏水；其余微孔中加入 50ul 的待测样本。
4. 加酶标溶液：标准品组、待测样本组各孔中加入 100ul 的酶标溶液（空白对照孔除外）。
5. 温育：酶标板用封板纸密封后，放入湿盒内于 37℃恒温孵育 1 小时。
6. 洗板：用稀释后的洗涤液注满每孔，静置 15-30s，充分清洗酶标板 5 次，用吸水纸彻底拍干。
7. 显色：各孔加入显色剂 A 液 50ul 后，再加入显色剂 B 液 50ul。
8. 终止：25-37℃下避光反应 10-15 分钟，加入 50ul 终止液。
9. 读板：在 450nm 波长读取各孔的 OD 值。

   注：读板时必须拭干板底残留的液体和手指痕迹，读板时间控制在终止反应后的 30 分钟内，以免影响准确性。

数据计算

1. 绘制标准曲线：各标准品 OD 值减去底色即减去空白孔 OD 值，以 6 个标准品的 OD 值为纵坐标 y，以标准品的浓度为横坐标 x，绘制曲线图，如图示，并计算出回归方程 y=ax+b。
2. 将待测样品的 OD 值代入方程式，计算各组样品浓度，再乘以稀释倍数，即为样品的实际 ATA 浓度。
3. 敏感度：1.0 pg/ml



注意事项：

1. 实验操作中必须使用一次性吸头，避免交叉污染。
2. 加样：加样时，要控制加样速度，避免第一孔与最后一孔间的时间间隔过大，否则将会导致不同的预孵育时间，从而影响实验的准确性以及重复性。
3. 孵育：严格按照说明书上规定的孵育时间和温度进行。
4. 反应时间的控制：加入底物后请定时观察反应孔的颜色变化，如果颜色较深，请提前加入终止液终止反应。
5. 建议实验前预测样品含量，如样品浓度过高，应对样品进行稀释，计算结果时乘以相应的稀释倍数。
6. 建议使用本试剂盒时先做预实验（即先做标准曲线，试用几个标本），如果对本试剂盒有任何疑问，可和所购经销商联系，如果因运输过程导致试剂盒失效，可要求调换，但概不承担产品本身以外的任何损失。

安全性

1. 避免人体直接接触显色剂A、B和终止液。一旦接触到这些液体，请尽快用水冲洗。
2. 实验中不要进食、抽烟或使用化妆品。
3. 不要用嘴吸取试剂盒里的任何成份。

保存条件及有效期：

1.试剂盒保存：2-8℃

2.有效期：6个月

Human Activin A（ATA）

Product Numbers: HEA002

ELISA Kit Instructions

Use:

This ELISA kit is for quantitative detecting Human Activin A (ATA) content in specimens of cells culture supernates, body fluids, tissues homogenate, serum, and plasma. The kit is for scientific research use only. Not for diagnostic or therapeutic procedures.

Principle:

This ATA enzyme linked immunosorbent assay employs the quantitative sandwich enzyme immunoassay technique. The microplate provided in this kit has been pre-coated with a monoclonal antibody specific for ATA. Standards or samples are then pipetted into the microplate wells, and ATA present in the samples or standards binds to antibodies adsorbed to the microplate wells. In order to quantitatively determine the amount of ATA present in the samples, the horseradish peroxidase (HRP)-conjugated polyclonal antibody specific for ATA are added to each well. The microplate is incubated for 1h, and then the wells are thoroughly washed to remove any unbound components. The substrate solution A and B is respectively added to each well. After the enzyme (HRP) and substrate reacting over a short period, this reaction is stopped by addition of a sulphuric acid solution and the color change is measured at a wavelength of 450 nm. The proportion to amount of ATA bound in the initial step develops in the color change.

Reagents in kit:

Numerical order	Name	Specification
1	Microplate	96 well（Disassemble plate）
2	Enzyme conjugate	10mlx 1 vial
3	Standard	1 ml x 6 vials
4	Lysis buffer solution	6 ml x 1 vial
5	Substrate A	6 ml x 1 vial
6	Substrate B	6 ml x 1 vial
7	Stop solution	6 ml x 1 vial
8	Washing solution（x 100）	50ml x1 vial
9	Plate sealing membrane	1
10	Instruction manual	1
Remarks	1. 1～6 Standard Concentrations：0、50、100、250、500、1000pg/ml 2. Lysis Buffer Solution is applicabled only when the sample is cell culture supernates, body fluids, tissues homogenate	

Experiment materials and reagent preparation:

1. Instruments and materials: microplate reader capable of measuring absorbance at 450 nm (preheat 30 minutes before use), micropipette, pipette tips, distilled water or deionized water, filter paper.
2. Lysis Buffer Solution: add 5uL of Lysis Buffer Solution into 50uL specimens, mix well and stand for one hour (This step is required when the sample is cells culture supernates, body fluids, tissues homogenate; if the sample is serum or blood plasma, then this step should be skipped. The proportion of Lysis buffer and Specimens shall be no less than 1:10).
3. Washing solution: prepare washing solution in the proportion of 1:100. Mix gently to avoid foaming.

Sample collection and storage:

1. Cell culture supernatants: After centrifugation at 1000xg for 15 minutes, collect supernatants with a sterile tube.
2. Cells: After washing cells 3 times with PBS, adjust the cell concentration to 10^4-10^6/ml; cells are broken down either through repeated freeze-thaw cycles or by using ultrasound treatment, and then cell ingredients are released. Collect the supernatants after centrifugation.
3. Serum: allow samples to clot before centrifugation for 15 minutes at 1000 x g, collect the supernatants.
4. Plasma: Collect plasma using EDTA or citric acid sodium as anticoagulation; after mixing, stand for 10 to 20 minutes, and then centrifuge at 1000 x g for 15 minutes, collect the supernatants.
5. Body Fluid: include fluid from thoracic or abdominal cavity, cerebrospinal fluid, secretion and so on. Collect supernatants with tubes (without pyrogen and endotoxin) after centrifugation at 1000 x g for 15 to 20 minutes.
6. Tissue samples: After weighing 0.5mg specimens and add into 500ul PBS, specimen homogenate can be prepared by manual grind, a homogenate device, or ultrasound. After centrifugation for 20 minutes (at 2000-3000 RPM), collect the supernatants.
7. Samples should not contain NaN3 because NaN3 inhibit horseradish peroxidase (HRP) activity.
8. Storage: Assay immediately or aliquot and store samples at -70℃, avoid repeated freeze-thaw cycles. Precipitation appears during storage, must centrifuge again. As far as possible, do not use blood samples with hemolysis or hyperlipid. Samples

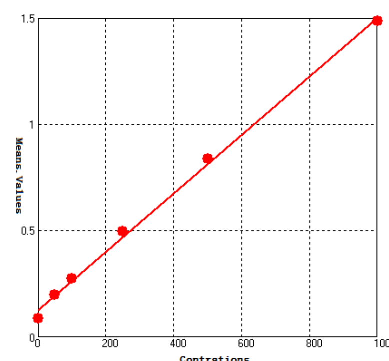
containing a visible precipitate must be clarified prior to use in the assay. Don't thaw specimens at 37 ℃ or higher. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Assay procedure:

1. The kit and samples are brought to room temperature (20-25℃) for 30 minutes before use.
2. Group: take out 96 microplate and determine microplate strips according to quantity of the specimens and the standard samples, and then remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack and reseal. Set up the standard group (six concentrations), blank control group, and the sample group respectively. To minimize the error and guarantee the accuracy, it is suggested that each sample is arranged with three or more wells.
3. Add specimens: in accordance with the order of the standard, add 50ul standard solution in each well of standard group; 50ul distilled water in each well of blank control group; and 50ul of the sample in the rest.
4. Add Enzyme conjugate: add 100ul of Enzyme conjugate to the standard group and specimen group respectively (with the exception of blank control group).
5. Incubation: Plates are sealed tightly with plate sealing membrane, incubated in wet box at 37 ℃ constantly for1 hour.
6. Wash plate: filling each well full with diluted washing solution, rest for 15-30 seconds, repeat for 5 times, thoroughly pat dry with absorbent paper.
7. Color development: to each well add Substrate A 50ul, followed by Substrate B 50ul.
8. Color termination: after reaction at 25-37 ℃ in dark for 10-15 minutes, add Stop solution 50uL to each well.
9. Read plate: Determine the optical density (OD) of each well within 30 minutes, using a microplate reader set to 450 nm. Please wipe away the residual liquid and fingers trace in advance

Data calculation

1. This standard curve is used to determine the amount of ATA in an unknown sample. The standard curve is generated by plotting the mean O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis.
2. First, calculate the mean O.D. for each standard and sample. All O.D. values, are subtracted by the mean value of the zero standard before result interpretation. Construct the standard curve using graph paper or statistical software.
3. To determine the amount in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration.
4. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.
5. The sensitivity by this assay is 1.0 pg/ml.
6. Standard curve



Note:

1. To avoid cross contamination, disposable tips should be used for one-time only.
2. Add samples: to minimize the interval between the first well and the last one, the speed of adding sample should be controlled. Otherwise, it will lead to difference of the incubation time, thus affecting the accuracy of the experiment.
3. Incubation: in strict accordance with the demands about the instruction of incubation time and temperature.
4. Control of the reaction time: monitor the color change in the well in fixed time after substrate is added, if the color is darker, please add Stop solution ahead of schedule.
5. It is suggested to get preliminary data before a formal test if the concentration of the specimen is too high. Dilute the specimen and times the results accordingly when calculation.
6. It is suggested to get a preliminary test done on the kit (namely get standard curve made first, trial on a few specimens), if there is any doubt about this kit, or the failure is resulted from transportation, please contact customer service of company or agent for replacement; but it shall not be subject to any loss related to the product itself.

Safety:

1. Avoid direct contact the human body with color-display reagent A, B and the terminating reaction solution. Once it happened, please wash out with water as soon as possible.
2. Don't eat or drink, smoke or make up during experiment.
3. Don't suck up any reagents in the kit with mouth.

Preservation conditions and validity:

1. Storage: 2-8 ℃
2. Period of Validity: six months