PRODUCT INFORMATION & MANUAL

Human Total ERα/NR3A1 Valukine™ ELISA

Catalog Number: VAL188

For the quantitative determination of natural and
recombinant human Total ERα/NR3A1 concentrations

For research use only.
Not for diagnostic or therapeutic procedures.

Bio-Techne China Co. Ltd
P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001
info.cn@bio-techne.com

Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version 202403.1

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I. BACKGROUND

ER alpha (Estrogen receptor alpha; also Estradiol receptor and NR3A1) is a member of nuclear hormone receptor superfamily (1). It contains a N-terminal domain, two transcriptional activation functions (AF-1, AF-2), a highly conserved DNA binding domain, hinge region, and a C-terminal ligand binding domain (2-4). The C-terminal domain of ERα is highly structured upon ligand binding and this domain is highly conserved across many species. The N-terminal domain is less structured and poorly conserved across species, and is activated by both ligand-dependent and ligand-independent mechanisms (4).

ER alpha is expressed in a wide variety of tissues such as bones, uterus, bladder, ovary, prostate, testis, epididymis, kidney, breast, heart, vessel wall, pituitary and hypothalamus (5-7). It is normally quiescent and bound to heat-shock proteins and immunophilins (1-2). Following ligand (estrogenic compound 17 beta-estradiol) binding, it becomes activated, either homodimerizes or heterodimerizes with ER beta (8). Though ERα function is strongly activated by ligand binding, ERα function is also regulated by posttranslational modifications (PTMs), most significantly by phosphorylation (9). ERα phosphorylation occurs at multiple sites, most located in the N-terminal domain, is regulated by ligand binding and by ligand-independent mechanisms such as peptide growth factor signaling (10).ERα phosphorylation sites contribute to regulation of multiple functional activities including hormone sensitivity, nuclear localization, DNA binding, protein/chromatin interactions, protein stability and gene transcription (11,12).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human Total ERα/NR3A1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human Total ERα/NR3A1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for human Total ERα/NR3A1 is pipetted into the wells. After washing away any unbound substances, Streptavidin-HRP is pipetted into the wells. Following a wash to remove any unbound reagent, TMB substrate (Chromogenic agent) is added to the wells and color develops in proportion to the amount of human Total ERα/NR3A1 bound in the initial step. The color development is stopped, and the intensity of the color is measured.

B. LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- This kit is suitable for cell culture supernates, human serum and cell lysate.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent (1×) and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

Three samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples were tested in twenty separate assays to assess inter-assay precision.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	90.1	23.1	5.9	91.4	23.4	5.9
Standard Deviation	2.7	0.8	0.2	3.2	0.8	0.3
CV%	3.0	3.3	4.2	3.5	3.6	5.4

B. RECOVERY

The recovery of human Total ERα/NR3A1 spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 84.7 to 98.5% with an average of 91.9%.

The recovery of human Total ERα/NR3A1 spiked to different levels throughout the range of the assay in human serum was evaluated. The recovery ranged from 95.1 to 109.3% with an average of 101.4%.

The recovery of human Total ERα/NR3A1 spiked to different levels throughout the range of the assay in cell lysate was evaluated. The recovery ranged from 94.8 to 109.6% with an average of 101.5%.

C. SENSITIVITY

The minimum detectable dose (MDD) of human Total ERα/NR3A1 is typically less than 0.78 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

D. CALIBRATION

This Kit is calibrated against a highly purified *E.coli*-expressed recombinant human ERα produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human Total ERα/NR3A1 and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	91.4	85.6-102.3
1:4	94.7	83.2 -100.2
1:8	100.7	91.9-110.6
1:16	98.9	87.8-109.2

F. SAMPLE VALUES

Serum – Four human serum samples were evaluated for the presence of Total ERα/NR3A1 in this assay. All samples measured ranged from 13.6 to 25.4 pg/mL with an average of 21.7 pg/mL..

Cell Lysates – MCF-7 (1 x 10^6 cells/mL) were cultured in DMEM/F12 supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured for 72 h. After the supernates were harvested, rinse cells three times with PBS, making sure to remove any remaining PBS after the third rinse. Solubilize cells at 1 x 10^7 cells/mL in Cell Lysis Buffer and allow samples to sit on ice for 15 minutes. Assay immediately or store at ≤ -70 °C. Before use, centrifuge samples at 2000 x g for 5 minutes, and transfer the supernate to a clean test tube. The level of human Total ERα/NR3A1 in cell lysates was assayed, and measured 1929.6 pg/mL.

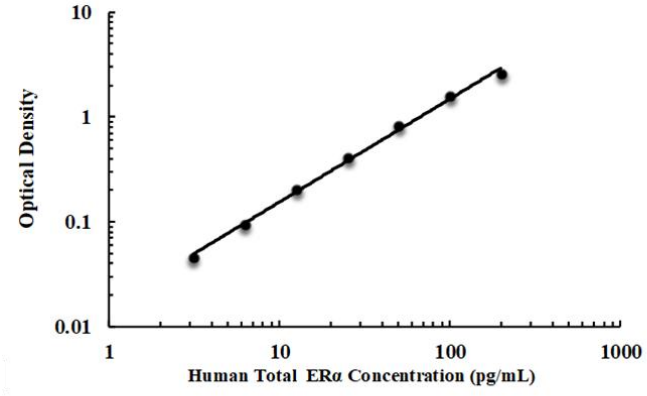
G. SPECIFICITY

The Human Total ERα/NR3A1 Valukine™ ELISA specifically recognizes ERα. Specificity was demonstrated by Western Blot analysis of the protein bound by the capture antibody supplied in the kit.

IV. EXPERIMENT

EXAMPLE STANDARD

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



pg/mL	OD	Average	Corrected
0	0.096 0.105	0.100	—
3.1	0.143 0.150	0.146	0.046
6.3	0.191 0.199	0.195	0.095
12.5	0.297 0.305	0.301	0.201
25	0.497 0.513	0.505	0.405
50	0.906 0.927	0.916	0.816
100	1.663 1.665	1.664	1.563
200	2.660 2.684	2.672	2.572

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human Total ERα/NR3A1 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human Total ERα/NR3A1.	1 plate
Human Total ERα/NR3A1 Standard	Recombinant human Total ERα/NR3A1 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Human Total ERα/NR3A1 Detection Antibody	Biotinylated Total ERα/NR3A1 antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Streptavidin-HRP A (200×)	200× Streptavidin conjugated to horseradish peroxidase.	1 vial
Calibrator Diluent (1×)	Buffered diluent used to dilute standard and samples.	2 vials
Detection Antibody Diluent Concentrate (5×)	A 5× concentrated buffered base used to dilute Detection Antibody.	1 vial
Cell Lysis Buffer (1×)	Buffered diluent used to lyse the cells. **Recommended:** Add 1µL PMSF (1000×) per 1 mL of Cell Lysis Buffer (1×) prior to use.	1 vial
Cell Diluent Concentrate (5×)	A 5× concentrated buffered base used to dilute Cell Lysis Buffer.	1 vial
PMSF (1000×)	Add 1µL PMSF (1000×) per 1 mL of Cell Lysis Buffer (1×) prior to use.	1 vial
Reagent Diluent Concentrate (10×)	A 10× concentrated buffered base used to dilute Detection Antibody and HRP.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution	1 vial
Stop Solution	2 N sulfuric acid.	1 vial
Plate Sealers	Adhesive strip.	3 strips

B. STORAGE

Unopened Kit	Store at 2-8 °C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Streptavidin-HRP A	May be stored for up to 1 month at 2-8 °C.*
	Wash Buffer (1×)	
	TMB Substrate	
	Stop Solution	
	Calibrator Diluent (1×)	
	Standard	Prepare fresh for each assay. Standards may be stored for up to 1 month at -20°C *.
	Detection Antibody	Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *
	Cell Diluent Concentrate (5×)	May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Cell Diluent (1×). Prepare fresh for each assay.
	Detection Antibody Diluent Concentrate (5×)	May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Detection Antibody Diluent (1×). Prepare fresh for each assay.
	Reagent Diluent Concentrate (10×)	May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Reagent Diluent (1×). Prepare fresh for each assay.
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*

Unopened Kit	Store at -20 °C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Cell Lysis Buffer (1×)	Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *
	PMSF (1000×)	

* Provided this is within the expiration date of the kit.

C. OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker capable of maintaining a speed of 500±50 rpm.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

Cell Lysates - Rinse cells three times with PBS, making sure to remove any remaining PBS after the third rinse. Solubilize cells at 1 x 10^7 cells/mL in Cell Lysis Buffer and allow samples to sit on ice for 15 minutes. Assay immediately or store at ≤ -70 °C. Before use, centrifuge samples at 2000 x g for 5 minutes, and transfer the supernate to a clean test tube. For assaying, dilute lysates 6-fold with Cell Diluent (1×), if continue to dilute, make further dilutions in Calibrator Diluent (1×).

B. SAMPLE PREPARATION

Cell culture supernate samples and human serum samples recommend a 2-fold dilution prior to the assay. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

Cell lysates samples recommend a 6-fold dilution prior to the assay. A suggested 6-fold dilution is 50 µL of sample + 250 µL of Cell Diluent (1×), if continue to dilute, make further dilutions in Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Note: *Bring all reagents to room temperature before use.*

Cell Lysis Buffer- Add 1µL PMSF (1000×) per 1 mL of Cell Lysis Buffer (1×) prior to use.

Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of

Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

Cell Diluent (1×) - Use deionized or distilled water to prepare Cell Diluent (1×).

Detection Antibody Diluent (1×) - Use deionized or distilled water to prepare Detection Antibody Diluent (1×).

Reagent Diluent (1×) - Use deionized or distilled water to prepare Reagent Diluent (1×).

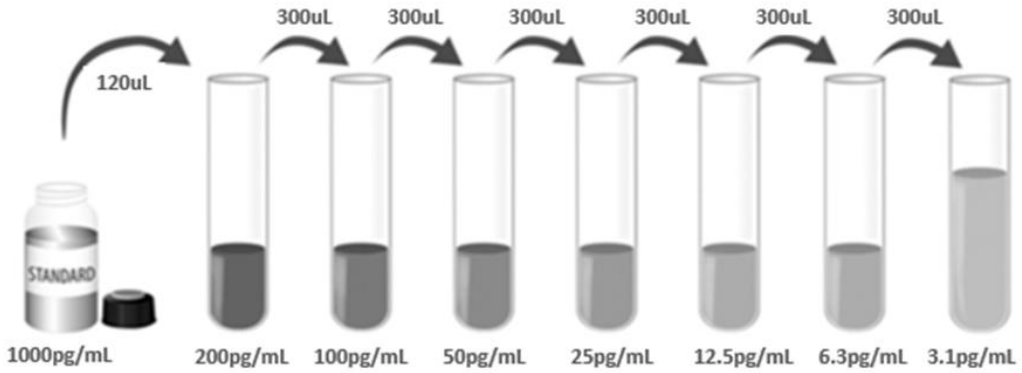
Detection Antibody (1×) - Centrifuge briefly before opening. Reconstitution volume refer to vial label to prepare Detection Antibody (100×). Allow the Detection Antibody to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Aliquot and store if needed. Dilute to Detection Antibody (1×) with Detection Antibody Diluent (1×). Prepare at least 15 minutes prior to use.

Streptavidin-HRP A (1×) - Centrifuge briefly before opening. Dilute to the working concentration specified on the vial label using Reagent Diluent (1×).

Human Total ERα/NR3A1 Standard - Centrifuge briefly before opening. Refer to the vial label for the reconstitution volume*. This reconstitution produces a stock solution of 1000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

*If you have any question, please seek help from our Technical Support.

Pipette 480 µL of Calibrator Diluent (1×) into the 200 pg/mL tube. Pipette 300 µL of the appropriate Calibrator Diluent (1×) into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 200 pg/mL standard serves as the high standard. The Calibrator Diluent (1×) serves as the zero standard (0 pg/mL).



D. TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- TMB substrate should remain colorless until added to the plate. Keep TMB Substrate protected from light. TMB Substrate should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the TMB substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB substrate.

VII.ASSAY PROCEDURE

Note: *Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.*

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of standard and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker set at 500±50rpm.** A plate layout is provided for a record of standards and samples assayed. (Samples may require dilution. See Sample Preparation section.)
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of the Detection Antibody (1×) diluted in Detection Antibody Diluent (1×), to each well. Cover with a new adhesive strip and **incubate for 2 hours at room temperature on a horizontal orbital microplate shaker set at 500±50rpm**.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of the working dilution of Streptavidin-HRP A to each well. Cover the plate and **incubate for 20 minutes at room temperature. Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of TMB Substrate to each well. **incubate for 20 minutes at room temperature. Protect from light.**
10. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm.

If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

12. CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human Total ERα/NR3A1 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

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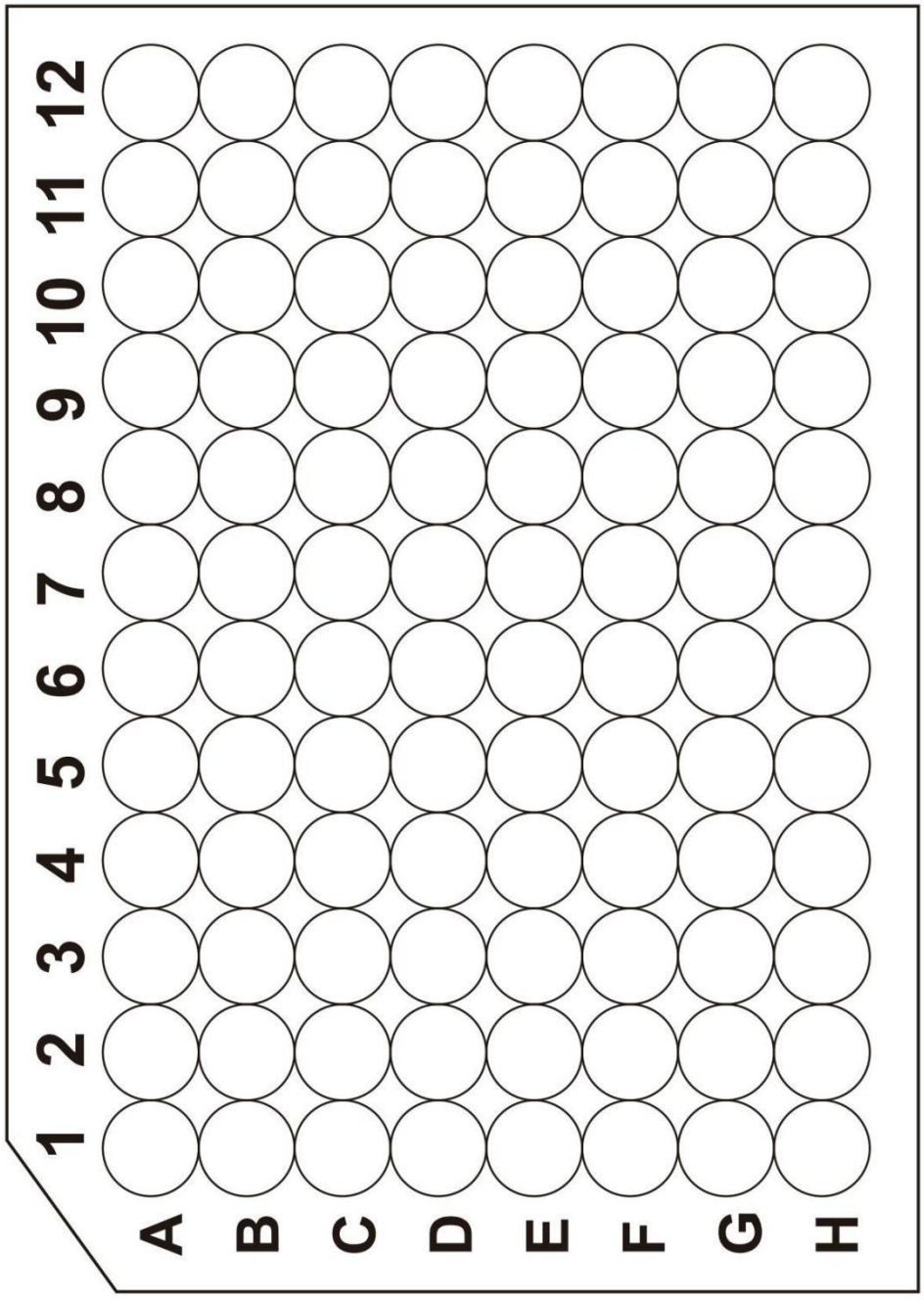
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 Total ERα /NR3A1 Valukine™ ELISA 试剂盒

目录号：VAL188

适用于定量检测天然和重组人 Total ERα/NR3A1 的浓度

科研专用，不可用于临床诊断

Bio-Techne China Co. Ltd

P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001

info.cn@bio-techne.com

有效期详见试剂盒包装标签

Novus 试剂盒确保在你收货日期 3 个月内有效

版本号 202403.1

目录

I. 背景

ER α(雌激素受体α；又称雌二醇受体和 NR3A1)是核激素受体超家族的成员(1)。它包含一个 N 端结构域，两个转录激活功能域(AF-1, AF-2)，一个高度保守的 DNA 结合域，铰链区和一个 C 端配体结合域(2-4)。ERα的 C 端结构域在配体结合的基础上高度结构化，该结构域在许多物种中高度保守。N 端在物种间的结构较差，保守性较差，它可以通过配体依赖和配体非依赖机制激活(4)。

ER α在多种组织中表达，如骨骼、子宫、膀胱、卵巢、前列腺、睾丸、附睾、肾脏、乳腺、心脏、血管壁、垂体和下丘脑(5-7)。它通常是静止的并与热休克蛋白和亲免疫蛋白结合(1-2)。在与配体(雌激素化合物 17 β -雌二醇)结合后，ER α被激活，与 ER β形成同源二聚体或异源二聚体(8)。虽然配体结合会强烈激活 ERα功能，但 ERα功能也受到翻译后修饰(PTMs)的调节，最主要的是磷酸化(9)。ERα的磷酸化发生在多个位点，大多数位于 N 端结构域，受配体结合和非配体机制的调节(10)。ERα磷酸化位点有助于调节多种功能活动，包括核定位、DNA 结合、蛋白质/染色质相互作用、蛋白质稳定性和基因转录等(11,12)。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人Total ERα/NR3A1抗体包被于微孔板上，样品和标准品中的Total ERα/NR3A1会与固定在板上的抗体结合，游离的成分被洗去；接着加入生物素化的抗人Total ERα/NR3A1检测抗体进行孵育，洗涤去除未结合的物质后，加入链霉亲和素标记的辣根过氧化物酶（Streptavidin-HRP）孵育。洗涤去除未结合的试剂后，加入TMB底物溶液（显色剂）。溶液颜色与结合的Total ERα/NR3A1的量成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- **仅供科研使用，不可用于体外诊断；**
- 该试剂盒适用于细胞培养上清样本，人血清样本和细胞裂解液样本；
- 请在试剂盒有效期内使用；
- 不同试剂盒及不同批号试剂盒的组分不能混用；
- 样本值若大于标准曲线的最高值，应将样本用标准品稀释液（1×）稀释后重新检测；
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的效期等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测20次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板中分别检测20次，以确定板间精确度。

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
Mean (pg/mL)	90.1	23.1	5.9	91.4	23.4	5.9
Standard Deviation	2.7	0.8	0.2	3.2	0.8	0.3
CV%	3.0	3.3	4.2	3.5	3.6	5.4

B. 回收率

在细胞培养基样本中掺入检测范围内不同水平的人Total ERα/NR3A1，测定其回收率。回收率范围在84.7-98.5%，平均回收率在91.9%。

在血清样本中掺入检测范围内不同水平的人Total ERα/NR3A1，测定其回收率。回收率范围在95.1-109.3%，平均回收率在101.4%。

在细胞裂解液样本中掺入检测范围内不同水平的人Total ERα/NR3A1，测定其回收率。回收率范围在94.8-109.6%，平均回收率在101.5%。

C. 灵敏度

人Total ERα/NR3A1的最低可测量（MDD）一般小于0.78 pg/mL。

MDD是根据20个重复的零标准品孔的吸光度值的平均值加两倍标准差计算得到的相对应浓度。

D. 校正

此ELISA试剂盒经由R&D Systems生产的大肠杆菌表达的高纯度重组人ERα蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的人Total ERα/NR3A1，然后用标准品稀释液（1×）将样本稀释到检测范围内，测定其线性。

Dilution	Average % of Expected	Range (%)
1:2	91.4	85.6-102.3
1:4	94.7	83.2 -100.2
1:8	100.7	91.9-110.6
1:16	98.9	87.8-109.2

F. 样本预值

血清样本 - 使用本试剂盒检测了4份人血清样本中Total ERα/NR3A1的含量。所有样本的检测值在 13.6 -25.4 pg/mL之间，平均值为21.7 pg/mL。

细胞裂解液 - MCF-7 (1 x 10^6 cells/mL) 培养于含有10%胎牛血清的DMEM/F12培养基中，加100 U/mL青霉素和100 µg/mL硫酸链霉素。细胞培养72h。收集细胞上清后，用PBS冲洗细胞3次，确保在第3次冲洗后去除剩余的PBS。将细胞溶解于1 × 10^7 cells/mL的细胞裂解缓冲液中，并使样品在冰上反应15分钟。立即测定或储存在≤-70℃中。使用前，样品在2000 × g 离心5分钟，并将裂解液上清转移到一个新的试管中。检测细胞裂解液中人Total ERα/NR3A1的含量，检测值为1929.6 pg/mL。

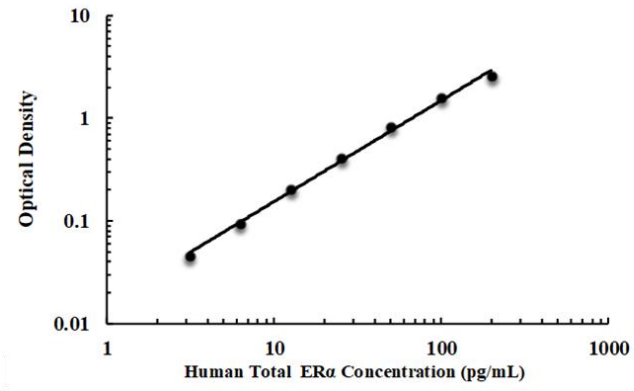
G. 特异性

人Total ERα/NR3A1 Valukine™ ELISA试剂盒特异性识别ERα。通过对试剂盒中提供的捕获抗体结合的蛋白进行Western Blot分析，证明其特异性。

IV. 实验

标准曲线实例

该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。



pg/mL	OD	Average	Corrected
0	0.096 0.105	0.100	—
3.1	0.143 0.150	0.146	0.046
6.3	0.191 0.199	0.195	0.095
12.5	0.297 0.305	0.301	0.201
25	0.497 0.513	0.505	0.405
50	0.906 0.927	0.916	0.816
100	1.663 1.665	1.664	1.563
200	2.660 2.684	2.672	2.572

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human Total ERα/NR3A1 Microplate	包被抗人 Total ERα/NR3A1 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Human Total ERα/NR3A1 Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Human Total ERα/NR3A1 Detection Antibody	生物素化的 Total ERα/NR3A1 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Streptavidin-HRP A (200×)	200×浓缩的链霉亲和素标记的 HRP	1 瓶
Calibrator Diluent (1×)	标准品稀释液用于稀释标准品和样本	2 瓶
Detection Antibody Diluent Concentrate (5×)	浓缩的检测抗体稀释液（5×）用于稀释检测抗体	1 瓶
Cell Lysis Buffer (1×)	细胞裂解液用于裂解细胞 **建议：**使用前，每 1 mL 细胞裂解液（1×）加 1μL PMSF (1000×)	1 瓶
Cell Diluent Concentrate (5×)	浓缩的细胞稀释液（5×）用于稀释细胞裂解液	1 瓶
PMSF (1000×)	使用前，每 1 mL 细胞裂解液（1×）加 1μL PMSF (1000×)	1 瓶
Reagent Diluent Concentrate (10×)	浓缩的试剂稀释液（10×）用于稀释检测抗体和 HRP	1 瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1 瓶
TMB Substrate	TMB ELISA 底物溶液 /TMB 底物溶液	1 瓶
Stop Solution	终止液	1 瓶
Plate Sealers	封板膜	3 张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="10">已打开，稀释或重溶的试剂</td><td>链霉亲和素-HRP A</td><td rowspan="5">2-8℃储存，最多 30 天*</td></tr>
<tr><td>洗涤液（1×）</td></tr>
<tr><td>TMB 底物溶液</td></tr>
<tr><td>终止液</td></tr>
<tr><td>标准品稀释液（1×）</td></tr>
<tr><td>标准品</td><td>使用时新鲜配制*
标准品-20℃储存，最多 30 天*</td></tr>
<tr><td>检测抗体</td><td>分装， -20℃储存，最多 30 天*</td></tr>
<tr><td>细胞稀释液（5×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×检测抗体稀释液，多余的丢弃</td></tr>
<tr><td>检测抗体稀释液（5×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×检测抗体稀释液，多余的丢弃</td></tr>
<tr><td>试剂稀释液（10×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×试剂稀释液，多余的丢弃</td></tr>
<tr><td>包被的微孔板条</td><td>将未用的板条放回带有干燥剂的铝箔袋内，密封：2-8℃储存，最多 30 天*</td></tr>
<tr><td>未开封试剂盒</td><td colspan="2">-20℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="2">已打开，稀释或重溶的试剂</td><td>细胞裂解液（1×）</td><td rowspan="2">分装， -20℃储存，最多 30 天*</td></tr>
<tr><td>PMSF（1000×）</td></tr>
</table>

*必须在试剂盒有效期内

C. 实验所需自备试验器材

- 酶标仪（可测量450 nm检测波长的吸收值及540 nm或570 nm校正波长的吸收值）
- 高精度加液器及一次性吸头
- 蒸馏水或去离子水
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机
- 500 mL量筒
- 振荡器（速度可调至500±50 rpm）

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。使用后请彻底洗手。

VI. 实验前准备

A. 样品收集及储存

细胞培养上清液：颗粒物应离心去除；立刻检测样本。样本收集后若不及时检测，需按一次使用量分装，冻存于≤-20℃冰箱内，避免反复冻融。样本可能需要用标准品稀释液（1×）稀释。

血清样本：用血清分离管(SST)分离血清。使血样室温凝集30分钟，然后1000 × g离心15分钟。吸取血清样本之后即刻用于检测，或者分装，≤-20℃贮存备用。避免反复冻融。样本可能需要用标准品稀释液（1×）稀释。

细胞裂解液：用PBS冲洗细胞三次，确保在第三次冲洗后去除任何剩余的PBS。将细胞溶解于1 × 10^7 cells/mL的细胞裂解液中，并使其在冰上反应15分钟。立即检测或保存在≤-70°C。使用前，将样品在2000 x g下离心5分钟，并将上清液转移到新的试管中。用于检测时，用细胞稀释剂(1×) 6倍稀释，如果继续稀释，则用标准品稀释剂(1×)进一步稀释。

B. 样本准备工作

细胞上清样本和人血清样本建议用标准品稀释液（1×）2倍稀释后进行检测，例如：100 µL样本溶液+100 µL标准品稀释液（1×）。最佳稀释度应由最终用户确定。

细胞裂解液样本建议用细胞稀释液（1×）6倍稀释后进行检测。建议用50 µL样品+ 250 µL细胞稀释液(1×)，若继续稀释，则用标准品稀释液(1×)进一步稀释。最佳稀释度应由最终用户确定。

C. 检测前准备工作

使用前请将所有试剂放置于室温

细胞裂解液：使用前，每1 mL细胞裂解液（1×）加1µL PMSF (1000×)。

洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用去离子水或蒸馏水稀释配制成500 mL工作浓度的洗涤液（1×）。

细胞稀释液（1×）：使用去离子水或蒸馏水稀释配制成细胞稀释液（1×）。

检测抗体稀释液（1×）：使用去离子水或蒸馏水稀释配制成检测抗体稀释液（1×）。

试剂稀释液（1×）：使用去离子水或蒸馏水稀释配制成试剂稀释液（1×）。

检测抗体（1×）：开盖前请瞬时离心。参考检测抗体瓶标签重溶冻干粉，制备检测抗体（100×）。轻轻震摇至少15分钟，其充分溶解。如有需要分装保存。用检测抗体稀释液

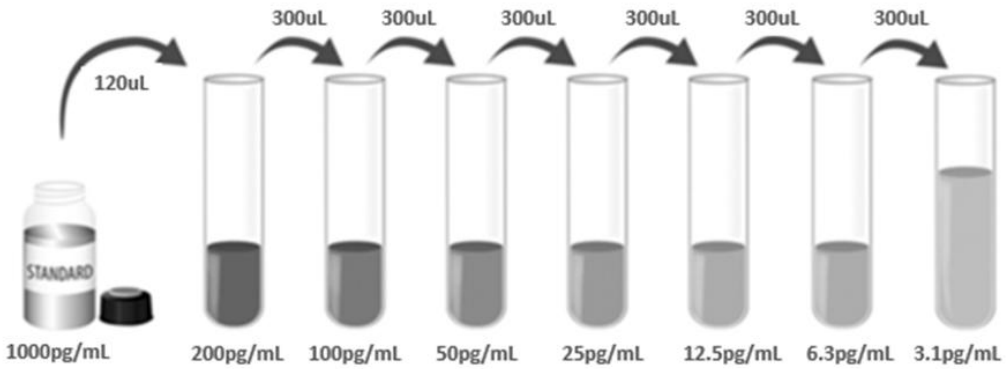
（1×） 稀释至检测抗体（1×），至少在使用前15分钟准备。

链霉亲和素- HRP A（1×）：开盖前请瞬时离心。用试剂稀释液（1×）将链霉亲和素- HRP A稀释至工作浓度。

人Total ERα/NR3A1 标准品：开盖前请瞬时离心。冻干标准品的重溶请参考瓶身标签。得到浓度为1000 pg/mL标准品母液。轻轻震摇至少15分钟，使其充分溶解。

*如有疑问，请咨询我们的技术支持。

向200 pg/mL稀释管中加入480 µL标准品稀释液（1×），其余各稀释管中加入300 µL标准品稀释液（1×）。将标准品母液参照下图做系列稀释，每管须充分混匀后再移液到下一管。200 pg/mL管作标准曲线最高点，标准品稀释液（1×）可用作标准品零点（0 pg/mL）。



D. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫；
- 为了避免交叉污染，配制不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽；
- 建议15分钟内完成一块板的上样；
- 每次孵育时，正确使用封板膜可保证结果的准确性；
- TMB底物溶液在上板前应为无色，请避光保存；加入微孔板后，TMB底物将由无色变成不同深度的蓝色；
- 终止液上板顺序应同TMB底物上板顺序一致；加入终止液后，孔内颜色由蓝变黄；若孔内有绿色，则表明孔内液体未混匀，请充分混合。

VII.操作步骤

使用前请将所有试剂和样本放置于室温，建议所有的实验样本和标准品做复孔检测

1. 按照上一节的说明，准备好所有需要的试剂和标准品；
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口；
3. 分别将不同浓度标准品和实验样本加入相应孔中，每孔100 µL。用封板膜封住反应孔，**室温500±50 rpm水平振荡孵育2小时。**说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置；（样本需要稀释，详情参见样本制备部分。）
4. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400 µL，然后将板内洗涤液吸去。重复操作3次，共洗4次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体；
5. 在每个微孔内加入100 µL配制好的检测抗体（1×）。用封板膜封住反应孔，**室温500±50 rpm水平振荡孵育2小时**；
6. 重复第4步洗板操作；
7. 在每个微孔内加入100 µL稀释好的链霉亲和素- HRP A工作液。用封板膜封住反应孔，**室温孵育20分钟，注意避光**；
8. 重复第4步洗板操作；
9. 在每个微孔内加入100 µL TMB底物溶液，**室温孵育20分钟，注意避光**；
10. 在每个微孔内加入50 µL终止液，请轻拍微孔板，使溶液混合均匀；
11. 加入终止液后10分钟内，使用酶标仪测量450 nm的吸光度值，设定540 nm或570 nm作为校正波长。如果波长校正不可用，以450 nm的读数减去540 nm或570 nm的读数。这种减法将校正酶标板上的光学缺陷。没有校正而直接在450 nm处进行的读数可能会更高且更不准确；
12. **计算结果：**将每个标准品和样品的复孔吸光值取平均值，然后减去零标准品平均OD值（O.D.），使用计算机软件作四参数逻辑（4-PL）曲线拟合创建标准曲线。另一替代方法是，通过绘制y轴上每个标准品的平均吸光值与x轴上的浓度来构建标准曲线，并通过图上的点绘制最佳拟合曲线。数据可以通过绘制人Total ERα/NR3A1浓度的对数与O.D.的对数来线性化，并且最佳拟合线可以通过回归分析来确定。该程序将产生足够但不太精确的数据拟合。

    如果样品被稀释，从标准曲线读取的浓度必须乘以稀释倍数。

VIII. 参考文献

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96 孔模板图

请使用 96 孔模板图来记录标准品及样本在板内的位置