PRODUCT INFORMATION & MANUAL

Human EGF Valukine™ ELISA

Catalog Number: VAL111

For the quantitative determination of natural and recombinant human EGF concentrations

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

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

Version 202606.5

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I. BACKGROUND

EGF (Epidermal Growth Factor; also Urogastrone) precursor is a 185 kDa group 1 member of the EGF family of growth factors (1-4). Group 1 members are molecules that bind to, and activate, the EGF receptor (EGF R). All EGF family members are synthesized as type I transmembrane (TM) proteins that are proteolytically cleaved to generate soluble forms. Human EGF is a fragment of a 1185 amino acid (aa) proform that contains a 1010 aa extracellular region, a 21 aa TM segment, and a 153 aa cytoplasmic domain. The proform extracellular domain (ECD) has three principal structural modules. There are nine class B LDL R repeats, one von Willebrand Factor A domain, and nine EGF-like repeats, the most membrane-proximal of which constitutes the mature 53 aa EGF molecule (aa's # 971-1023 of the preproprecursor) (5, 6). The transmembrane 185 kDa EGF proform undergoes proteolytic processing to generate multiple isoforms. Cleavage by ADAM10 releases a 160-170 kDa isoform (aa 21-1023) that is found in most body fluids (7-10). This is accompanied by the appearance of numerous 40-100 kDa fragments that may represent proteolytic degradation products (10). The process that generates the 6 kDa mature EGF molecule is unclear. It may arise internally, or be generated on the cell surface through the action of membrane-bound serine proteases that act on either the solubilized, 160 kDa proform, or a 70 kDa processed form of the 160 kDa proform (11, 12).

Notably, and in addition to mature EGF, both the 185 kDa TM, and proteolytically cleaved (but unprocessed) circulating, 160 kDa proform have bioactivity (7, 13, 14). The activity in both cases is attributed to the sole EGF peptide embedded in the precursor. None of the accompanying EGF-like motifs have activity on the EGF R (15). There are four potential alternative splice forms for the gene encoding EGF, none of which affect the mature EGF sequence. Two are in the ECD and show deletions of aa 913-953 and aa 314-355, respectively. Two others are in the cytoplasmic region and contain substitutions of 12 aa and 17 aa for aa 1125-1207 and aa 1136-1207, respectively. Mature human EGF is 70%, 70%, and 85% aa identical to mouse, rat and porcine EGF, respectively. Cells known to express EGF include platelets (16), cerebral neurons, astrocytes, and cerebellar Purkinje cells (3), cells of the Brunner (duodenum) and submandibular glands (17), nonpigmented ciliary epithelium (18), and cells of the anterior pituitary (19).

EGF has a number of diverse physiological effects. A full appreciation of its activity is complicated by the fact that it operates through the EGF receptor, which is utilized by other EGF family members, heterodimerizes with other EGF R family members, and associates with other transmembrane proteins such as the PDGF Rβ and HGF receptor (16, 20, 21). In any event, EGF is proposed to affect both fetal and adult tissues. In the fetus, EGF influences thymocyte growth and differentiation at the double negative-to-double positive stage (22). It also seems to drive neuroglia production at the expense of neuron formation (3), and promote epithelization (23). Finally, it inhibits adipocyte maturation, thus increasing preadipocyte numbers (23). In the adult, EGF plays a role in mammary gland lactogenesis (24). It also causes fibroblast mitosis, ECM dissociation, and migration, general effects often associated growth factor activity (25).

The ligand-binding receptor for EGF is the EGF receptor (also known as HER1 and ErbB1) (16). Although uncertainty exists as to the exact mechanism for receptor activation, it is now suggested that one EGF molecule binds to one receptor molecule at two distinct sites. This forces a conformational change in the receptor that allows for its association with a second EGF-EGF R complex (26). This dimerization forms a functional EGF receptor. It is also known that ErbB2 heterodimerizes with EGF R, but ErbB2 does not itself bind EGF. This may be due to the fact that ErbB2 exists naturally in a form that will form a dimer, but contains a ligand-binding site that is inaccessible to ligands. Thus, it waits for an activated partner (EGF-EGF R) before it forms a functional EGF receptor (26). ErbB2:ErbB2 homodimers are precluded from forming due to an inherent electrostatic repulsion. EGF is also suggested to participate in ErbB3:ErbB2 heterodimer formation at high concentrations (27). The significance of this is unknown. Alternative splice forms of EGF R exist in tumor cells, and may contribute to either tumorigenesis or to sensitivity to EGF R inhibitors (28).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human EGF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human EGF present is bound by the immobilized antibody. After washing away any unbound substances, an antibody conjugated to horseradish peroxidase for human EGF is added to the wells. Following a wash to remove any unbound reagent, TMB substrate solution (Chromogenic agent) is added to the wells and color develops in proportion to the amount of human EGF 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 supernate and human serum, human platelet-poor plasma, human saliva, human urine.
- 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×)/RD6N 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 of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

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

CELL CULTURE SUPERNATE/URINE ASSAY

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
N	20	20	20	20	20	20
Mean (pg/mL)	17.8	53.1	97.8	18.4	52.1	90.3
Standard Deviation	0.519	1.12	2.08	1.02	1.53	3.74
CV%	2.9	2.1	2.1	5.5	2.9	4.1

SERUM/PLASMA/SALIVA ASSAY

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
N	20	20	20	20	20	20
Mean (pg/mL)	27.3	86.1	162	28.7	86.3	159
Standard Deviation	1.34	2.92	6.40	1.75	5.88	12.6
CV%	4.9	3.4	3.9	6.1	6.8	7.9

B. RECOVERY

The recovery of human EGF spiked to three different levels in samples throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range (%)
Cell culture media (n=4)	99	96 - 103
Serum* (n=4)	103	97 - 108
Platelet-poor EDTA plasma (n=4)	97	88 - 102
Platelet-poor heparin plasma (n=4)	95	88 - 102
Platelet-poor citrate plasma (n=4)	101	96 - 107
Urine* (n=4)	97	91 - 106

*Samples were diluted prior to assay as directed in the Sample Preparation section.

C. SENSITIVITY

Thirty-five assays were evaluated and the minimum detectable dose (MDD) of human EGF ranged from 0.089-0.740 pg/mL. The mean MDD was 0.266 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 immunoassay is calibrated against a recombinant human EGF 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 EGF and diluted with Calibrator Diluent (1×)/RD6N to produce samples with values within the dynamic range of the assay.

*Samples were diluted prior to assay.

		Cell culture media (n=4)	Serum (n=4)	Platelet-poor			Saliva* (n=4)	Urine* (n=4)
				EDTA plasma (n=4)	Heparin plasma (n=4)	Citrate plasma (n=4)		
1:2	Average % of Expected	102	100	105	106	103	102	98
	Range (%)	100-104	97-102	100-111	103-110	101-104	99-105	93-103
1:4	Average % of Expected	105	103	108	111	104	102	102
	Range (%)	102-108	99-109	101-112	107-115	99-108	98-107	100-104
1:8	Average % of Expected	107	107	107	113	108	101	97
	Range (%)	103-111	101-111	97-115	111-115	103-111	98-106	85-102
1:16	Average % of Expected	107	107	106	115	109	99	97
	Range (%)	100-113	99-116	93-117	111-119	102-114	96-104	85-103

F. SAMPLE VALUES

Serum/Plasma/Saliva/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human EGF in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Platelet-poor EDTA plasma (n=37)	4.87	5	ND-5.20
Platelet-poor heparin plasma (n=37)	4.81	16	ND-5.43
Platelet-poor citrate plasma (n=37)	ND	0	_____

ND=Non-detectable

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=37)	409	106-1063	151
Saliva (n=14)	1703	81.8-9600	2400

Sample Type	Mean (µg/g Creatinine)	Range (µg/g Creatinine)	Standard Deviation (µg/g Creatinine)
Urine (n=11)	23,019	12,352-36,894	8524

Cell Culture Supernates - Human peripheral blood mononuclear cells (PBL; 1×10^6 cells/mL) were cultured in RPMI 1640 supplemented with 10% fetal calf serum. Cells were cultured and stimulated with 10 µg/mL PHA for 6 days. An aliquot of the cell culture supernate was removed, assayed for levels of human EGF, and measured 5.4 pg/mL.

G. SPECIFICITY

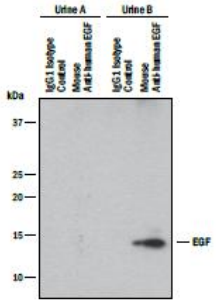
This assay recognizes both natural and recombinant human EGF.

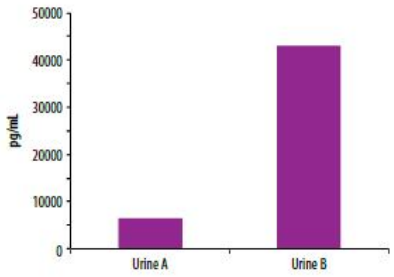
The factors listed below were prepared at 50 ng/mL in Calibrator Diluent (1×)/RD6N and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rhEGF control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant Human		Recombinant Mouse	
Amphiregulin	HB-EGF	Amphiregulin	Epigen
Betacellulin	MFG-E8	Betacellulin	Epiregulin
Cripto-1	NRG1/HRG1	Cripto	ErbB3
EGF R	NRG1 Isoform SMDF	EGF	ErbB4
Epigen	NRG1-α/HRG1-α EGF Domain	pro-EGF	MFG-E8
Epiregulin	NRG1-β1/HRG1-β1 EGF Domain	EGF R	
ErbB2	NRG1-β1/HRG1-β1 ECD		
ErbB3	TGF-α		
ErbB4	TNF-α		

Recombinant rat EGF does not interfere but does cross-reacts approximately 1.0% in this assay.

Recombinant human pro-EGF does not interfere but does cross-reacts approximately 1.3% in this assay, calculated by mass, and approximately 25%, calculated by molarity.





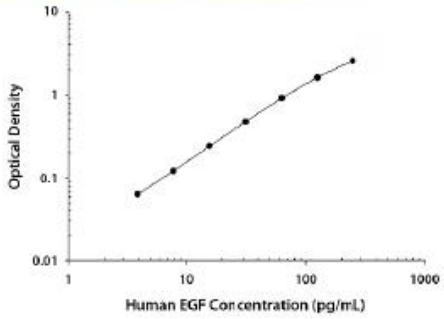
Human urine samples were analyzed by Immunoprecipitation/Western Blot and Valukine™ ELISA. Immunoprecipitated samples were resolved under reducing SDS-PAGE conditions, transferred to PVDF membrane, and immunoblotted with the detection antibody used in this kit. The Immunoprecipitation/Western Blot shows direct correlation with the ELISA value for these samples.

IV. EXPERIMENT

EXAMPLE STANDARD

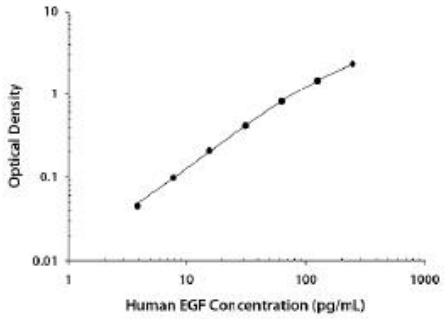
The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.010 0.012	0.011	—
3.91	0.073 0.079	0.076	0.065
7.81	0.132 0.135	0.134	0.123
15.6	0.247 0.262	0.255	0.244
31.3	0.474 0.496	0.485	0.474
62.5	0.918 0.926	0.922	0.911
125	1.612 1.627	1.620	1.609
250	2.537 2.599	2.568	2.557

SERUM/PLASMA/SALIVA



(pg/mL)	O.D.	Average	Corrected
0	0.007 0.008	0.008	—
3.91	0.052 0.054	0.053	0.045
7.81	0.103 0.109	0.106	0.098
15.6	0.212 0.216	0.214	0.206
31.3	0.417 0.421	0.419	0.411
62.5	0.804 0.823	0.814	0.806
125	1.470 1.492	1.481	1.473
250	2.347 2.353	2.350	2.342

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Amount
Human EGF Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human EGF	1 plate
Human EGF Conjugate	A solution of antibody against EGF conjugated to horseradish peroxidase	1 vial
Human EGF Standard	Recombinant human EGF in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	2 vials
Assay Diluent RD1-6	A buffered protein base with preservatives. May contain a precipitate. *For serum/plasma/saliva samples.*	1 vial
Calibrator Diluent (5×)/RD5E	A 5× concentrated buffered diluent protein base with preservatives. *For cell culture supernate/urine samples.*	1 vial
Calibrator Diluent RD6N	Animal serum with preservatives. *For serum/plasma/ saliva samples.*	2 vials
Wash Buffer concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives	1 vial
TMB Substrate	TMB ELISA Substrate Solution	2 vials
Stop Solution	2 N sulfuric acid	1 vial
Plate Covers	adhesive strip	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8°C not use past kit expiration date.</td></tr>
<tr><td rowspan="7">Opened/ Reconstituted Reagents</td><td>Wash buffer (1×)</td><td rowspan="4">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Standard</td><td>Use a new standard for each assay. Discard after use.</td></tr>
<tr><td>Assay Diluent RD1-6</td><td rowspan="2">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent RD6N</td></tr>
<tr><td>Calibrator Diluent (5×)/RD5E</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Microplate Wells</td><td>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.*</td></tr>
</table>

* 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.

D. PRECAUTION

- EGF is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.
- Some components in this kit contain ProClin 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

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 × 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 RD6N.

Platelet-poor Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. An additional centrifugation step of the plasma at 10,000 x g for 10 minutes at 2-8°C is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤-20°C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD6N.

EGF is present in platelet granules and is released upon platelet activation. Therefore, to measure circulating levels of EGF, platelet-poor plasma should be collected for measurement. It should be noted that many protocols for plasma preparation, including procedures recommended by the Clinical and Laboratory Standards Institute (CLSI), result in incomplete removal of platelets from blood.

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤-20°C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD6N.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter. Assay immediately or aliquot and store at ≤-20°C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

B. SAMPLE PREPARATION

Serum samples require at least a 20-fold dilution. A suggested 20-fold dilution is 25 µL of sample + 475 µL of Calibrator Diluent RD6N. Optimal dilutions should be determined by the end user.

Saliva samples require at least a 10-fold dilution due to endogenous levels. A suggested 10-fold dilution is 50 µL of sample + 450 µL of Calibrator Diluent RD6N. Optimal dilutions should be determined by the end user.

Urine samples require at least a 200-fold dilution. A suggested 200-fold dilution can be achieved by adding 25 µL of sample to 225 µL of Calibrator Diluent (1×)*. Complete the 200-fold dilution by adding 50 µL of the diluted sample to 950 µL of Calibrator Diluent (1×)*. Optimal dilutions should be determined by the end user.

*See Reagent Preparation section.

C. REAGENT PREPARATION

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

Note: *High concentrations of EGF are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

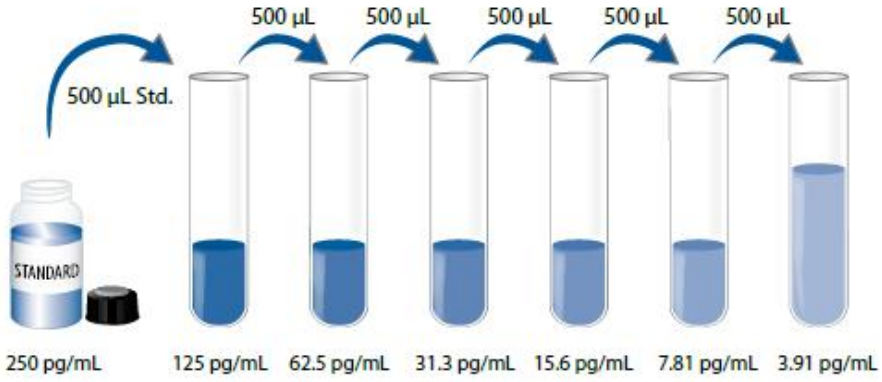
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

Calibrator Diluent (1×) - Use deionized or distilled water to prepare Calibrator Diluent (1×). Add 10 mL of Calibrator Diluent (5×) /RD5E to 40 mL of deionized or distilled water to prepare 50 mL of Calibrator Diluent (1×).

Human EGF Standard - Centrifuge briefly before opening. Refer to the vial label for reconstitution volume*. Reconstitute the Human EGF Standard with Calibrator Diluent (1×) (*for cell culture supernate/urine samples*) or Calibrator Diluent RD6N (*for serum/plasma/saliva samples*). This reconstitution produces a stock solution of 250 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 500 µL of the Calibrator Diluent (1×) (*for cell culture supernate/urine samples*) or Calibrator Diluent RD6N (*for serum/plasma/saliva samples*) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 250 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent (1×) /RD6N 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 Solution protected from light. TMB Substrate Solution 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

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

Note: *EGF is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents, samples 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. **For Serum/Plasma/Saliva Samples only:** Add 50 µL of Assay Diluent RD1-6 to each well. Assay Diluent RD1-6 may contain a precipitate. *Mix well before and during use.*
4. Add 200 µL of Standard or sample* per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature**. A plate layout is provided for a record of standards and samples assayed.
5. Aspirate each well and wash, repeating the process twice for a total of three washes. Wash by filling each well with Wash Solution (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.
6. Add 200 µL of human EGF Conjugate to each well. Cover with a new adhesive strip.

   For Cell Culture Supernate/Urine Samples: Incubate for 1 hour at room temperature.

   For Serum/Plasma/Saliva Samples: Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of TMB Substrate to each well. **Incubate for 20 minutes at room temperature. Protect from light**.

9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

10. 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.

11. **CALCULATION OF RESULTS**

    Average the duplicate readings for each standard and sample and subtract the average zero standard optical density. 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 EGF 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.

    *Samples may require dilution. See Sample Preparation section.

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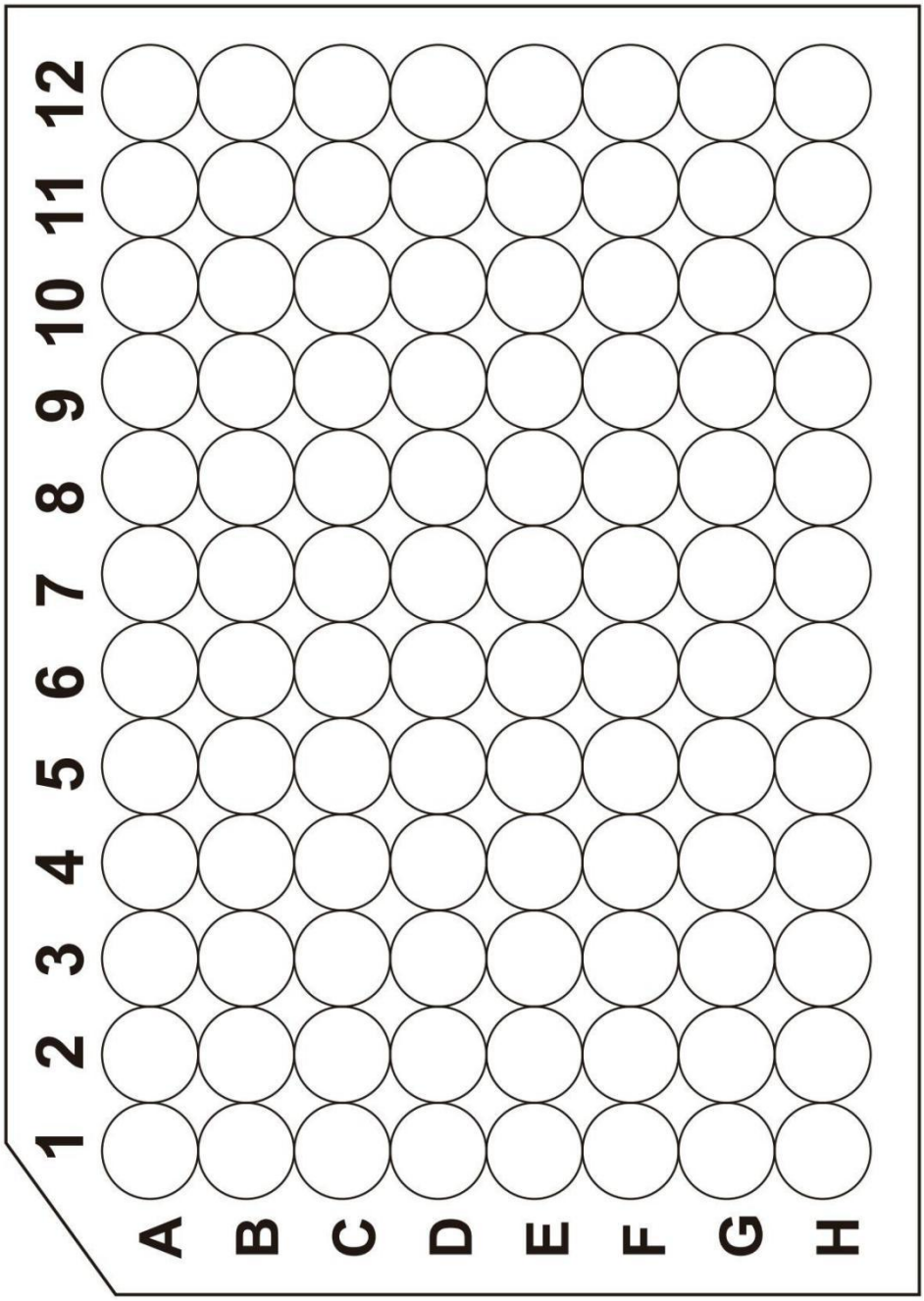
26.Burgess, A.W. et al. (2003) Mol. Cell 12:541.

27.Pinkas-Kramarski, R. et al. (1998) Oncogene 16:1249.

28.Albitar, L. et al. (2010) Mol. Cancer 9:116.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 EGF Valukine™ ELISA 试剂盒

目录号：VAL111

适用于定量检测天然和重组人表皮生长因子 EGF 的含量

科研专用，不可用于临床诊断

Bio-Techne China Co. Ltd

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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 202606.5

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I. 背景

EGF（表皮生长因子，又称Urogastrone）前体是生长因子EGF家族的Group 1成员，分子量为185 kDa（1-4）。Group I成员是结合并活化表皮生长因子受体（EGF R）的分子。EGF家族成员被合成为I型跨膜（TM）蛋白，且由蛋白水解生成可溶形式。人表皮生长因子（EGF）是一个长为1185个氨基酸前体分子形式的一个片段，包含1010个氨基酸细胞外区域、21个氨基酸TM区域和153个氨基酸胞质域。这个前体的胞外域（ECD）有三个主要的结构模块。其中包括9个BLDLR重复、1个血管性血友病因子A区域和9个类EGF重复，并在近膜区包含了成熟EGF分子的53个氨基酸的结构（为前体形式的第971位至1023位氨基酸）（5, 6）。这个跨膜185 kDa的亚型（氨基酸21-1023），存在于大多数体液中（7-10）。这个过程同时产生了大量40-100 kDa的蛋白片段，可能为蛋白水解降解产物（10）。6 kDa成熟EGF亚型的产生过程尚不清楚。它可能源自细胞内部，也可能源于细胞表面，通过细胞表面的膜结合丝氨酸酶水解可溶性的160 kDa前体或这个前体水解以后生产的70 kDa的分子亚型（11, 12）而来。

值得注意的是，成熟形式的EGF、185 kDa跨膜蛋白、已被水解但尚未加工的循环型分子和160 kDa前体分子都具有生物学活性（7, 13, 14）。而这些生物学活性主要是因为有EGF肽段嵌入在这些前体分子中。其它类型EGF的修饰都不具备结合EGF受体的活性（15）。编码EGF的基因在表达EGF蛋白过程中存在4种可能的剪切体，但都不影响成熟EGF的序列。其中两种剪切体发生在胞外区序列，分别删除了第913位至953位的氨基酸和第314至355位的氨基酸。另外两种剪切体则涉及EGF的胞内区域，分别以12个氨基酸替换了第1125位至1207位的氨基酸和以17个氨基酸替换了第1136-1207位的氨基酸。成熟的人EGF与小鼠、大鼠和猪的EGF的同源性分别是70%、70%和85%。已知表达EGF的细胞包括血小板（16）、大脑神经元、星状胶质细胞和小脑浦肯野细胞（3）、布鲁纳细胞（十二指肠）和颌下腺细胞（17）、不着色的纤毛上皮（18）和垂体前叶细胞（19）。EGF有许多不同的生理作用。目前广泛认可的EGF功能主要基于 EGF受体-配体的结合而实现。而EGF受体也能和其它EGF家族的蛋白结合，进而形成异源多聚体并与其它EGF受体家族成员二聚化，或者和其它跨膜蛋白如PDGF Rβ和 HGF受体产生关联（16, 20, 21）。在任何一种情况下，EGF都可以对胎儿和成人组织产生作用。在胎儿，EGF影响双阴性-双阳性阶段的胸腺细胞生长和分化（22）。在神经元形成过程中，EGF似乎也可以刺激神经胶质神经元的生成（3）并促进其上皮化（23）。最后，它能抑制脂肪细胞的成熟，从而增加前脂肪细胞数量（23）。在成人，EGF可以在乳腺的乳生成过程中发挥作用（24），也可使纤维细胞有丝分裂，ECM离解和迁移，广泛地影响生长因子相关的各种作用（25）。

EGF受体即表皮生长因子受体（也称为HER1和ErbB1）（16），其激活机制尚不明确。目前的研究认为每一个EGF分子和一个EGF受体分子有两个结合位点，这会迫使受体产生构象变化，而和第二个EGF-EGF R复合物结合（26）。这种二聚体即具有实际功能的EGF受体。ErbB2也可以和EGF受体形成异源二聚体，但不能和EGF结合。这可能是因为ErbB2天然以二聚体的形式存在，掩盖了其配体结合的位点，而使配体无法与其结合。因此，它需要结合一个已经激活的EGF-EGF R复合体才能形成一个功能性的EGF受体（26）。而由于内在的静电斥力作用，ErbB2自身无法形成同源二聚体。EGF也只有在高浓度的环境下也能和ErbB3：ErbB2形成异源多聚体（27）。这个过程的重要性，目前仍是未知的。EGF R的选择性剪切亚型存在于肿瘤细胞中，并可能参与肿瘤发生或使细胞对EGF受体抑制剂敏感（28）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人EGF抗体包被于微孔板上，样品和标准品中的人EGF会与固定在板上的抗体结合，游离的成分被洗去；加入辣根过氧化酶偶联的抗人EGF抗体孵育，洗涤去除未结合的物质后；加入TMB底物溶液（显色剂），溶液颜色与结合的目标蛋白成正比；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和人血清、人贫血小板血浆、人唾液、人尿液样本；
- 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×）/RD6N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，在不同板间分别检测20次，以确定板间精确度。

细胞培养上清/尿液

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
数量	20	20	20	20	20	20
平均值 (pg/mL)	17.8	53.1	97.8	18.4	52.1	90.3
标准差	0.519	1.12	2.08	1.02	1.53	3.74
CV%	2.9	2.1	2.1	5.5	2.9	4.1

血清/血浆/唾液

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
数量	20	20	20	20	20	20
平均值 (pg/mL)	27.3	86.1	162	28.7	86.3	159
标准差	1.34	2.92	6.40	1.75	5.88	12.6
CV%	4.9	3.4	3.9	6.1	6.8	7.9

B. 回收率

在样本中掺入检测范围内三个水平的人EGF，测定其回收率。

样品类型	平均回收率（%）	范围（%）
细胞培养基（n=4）	99	96 - 103
血清*（n=4）	103	97 - 108
贫血小板EDTA血浆（n=4）	97	88 - 102
贫血小板肝素血浆（n=4）	95	88 - 102
贫血小板枸橼酸盐血浆（n=4）	101	96 - 107
尿液*（n=4）	97	91 - 106

*按照“样品制备”部分的说明，在检测前对样品进行了稀释。

C. 灵敏度

对三十五项检测进行了评估，人EGF的最低可测剂量（MDD）一般在0.089-0.740 pg/mL范围内，MDD平均值为0.266 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 生产的重组人EGF蛋白校正。

E. 线性

不同的样本中含有或掺入高浓度的人EGF，然后用标准品稀释液将样本稀释到检测范围内，测定其线性。

		细胞培养基 (n=4)	血清 (n=4)	贫血小板			唾液* (n=4)	尿液* (n=4)
				EDTA血浆 (n=4)	肝素血浆 (n=4)	枸橼酸盐血浆 (n=4)		
1:2	平均值/期待值（%）	102	100	105	106	103	102	98
	范围（%）	100-104	97-102	100-111	103-110	101-104	99-105	93-103
1:4	平均值/期待值（%）	105	103	108	111	104	102	102
	范围（%）	102-108	99-109	101-112	107-115	99-108	98-107	100-104
1:8	平均值/期待值（%）	107	107	107	113	108	101	97
	范围（%）	103-111	101-111	97-115	111-115	103-111	98-106	85-102
1:16	平均值/期待值（%）	107	107	106	115	109	99	97
	范围（%）	100-113	99-116	93-117	111-119	102-114	96-104	85-103

*在检测前对样品进行了稀释。

F. 样本预值

血清/血浆/唾液/尿液 - 在本检测中，对来自表面健康志愿者的样品进行了人EGF存在性的评估。本研究中所用捐献者的病史资料不详。

样本类型	可检测均值（pg/mL）	可检测（%）	范围（pg/mL）
贫血小板EDTA血浆（n=37）	4.87	5	ND-5.20
贫血小板肝素血浆（n=37）	4.81	16	ND-5.43
贫血小板枸橼酸盐血浆（n=37）	ND	0	———

ND=未检测到

样本类型	均值（pg/mL）	范围（pg/mL）	标准差（pg/mL）
血清（n=37）	409	106-1063	151
唾液（n=14）	1703	81.8-9600	2400

样本类型	均值（μg/g 肌酐）	范围（μg/g 肌酐）	标准差（μg/g 肌酐）
尿液（n=11）	23,019	12,352-36,894	8524

细胞上清样本 - 人的外周血单核细胞（PBL；1×10^6细胞/mL）培养于含有10%胎牛血清的RPMI 1640培养基中，加10 μg/mL PHA刺激细胞6天。取细胞上清液测定人EGF含量，结果为5.4 pg/mL。

G. 特异性

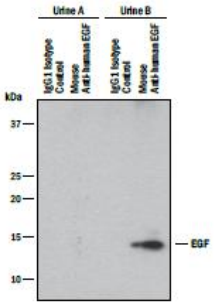
此ELISA法可检测天然及重组人EGF蛋白。

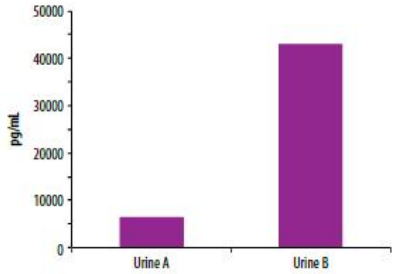
将以下因子用标准品稀释液（1×）/RD6N配制成50 ng/mL 的浓度来检测与人EGF的交叉反应。将50 ng/mL的干扰因子掺入中间范围的重组人EGF对照品中，来检测对人EGF的干扰。没有观察到明显的交叉反应或干扰。

Recombinant Human		Recombinant Mouse	
Amphiregulin	HB-EGF	Amphiregulin	Epigen
Betacellulin	MFG-E8	Betacellulin	Epiregulin
Cripto-1	NRG1/HRG1	Cripto	ErbB3
EGF R	NRG1 Isoform SMDF	EGF	ErbB4
Epigen	NRG1-α/HRG1-α EGF Domain	pro-EGF	MFG-E8
Epiregulin	NRG1-β1/HRG1-β1 EGF Domain	EGF R	
ErbB2	NRG1-β1/HRG1-β1 ECD		
ErbB3	TGF-α		
ErbB4	TNF-α		

重组大鼠 EGF 在本测定中不产生干扰，但确实存在约 1.0%的交叉反应。

重组人 pro-EGF 在本测定中不产生干扰，但确实存在交叉反应：按质量计算约为 1.3%，按摩尔浓度计算约为 25%。





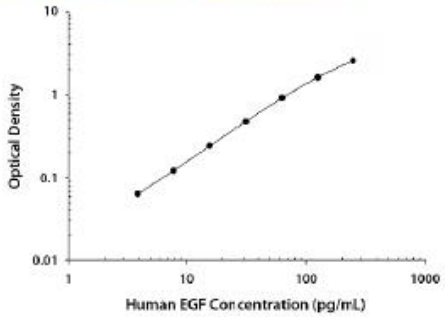
采用免疫沉淀/蛋白免疫印迹法（Immunoprecipitation/Western Blot）和Valukine™ ELISA试剂盒对人尿液样本进行了分析。免疫沉淀后的样本在还原性SDS-PAGE条件下进行分离，转移至PVDF膜，并使用本试剂盒中的检测抗体进行免疫印迹。免疫沉淀/蛋白免疫印迹的结果与这些样本的ELISA测定值呈直接相关性。

IV. 实验

标准曲线实例

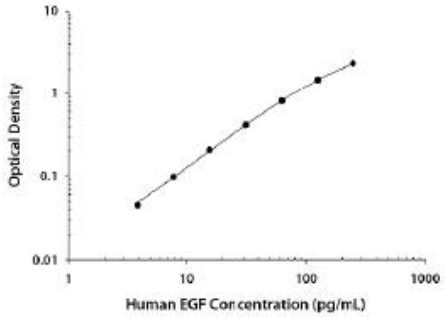
该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。

CELL CULTURE SUPERNATE/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.010 0.012	0.011	—
3.91	0.073 0.079	0.076	0.065
7.81	0.132 0.135	0.134	0.123
15.6	0.247 0.262	0.255	0.244
31.3	0.474 0.496	0.485	0.474
62.5	0.918 0.926	0.922	0.911
125	1.612 1.627	1.620	1.609
250	2.537 2.599	2.568	2.557

SERUM/PLASMA/SALIVA



(pg/mL)	O.D.	Average	Corrected
0	0.007 0.008	0.008	—
3.91	0.052 0.054	0.053	0.045
7.81	0.103 0.109	0.106	0.098
15.6	0.212 0.216	0.214	0.206
31.3	0.417 0.421	0.419	0.411
62.5	0.804 0.823	0.814	0.806
125	1.470 1.492	1.481	1.473
250	2.347 2.353	2.350	2.342

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human EGF Microplate	包被小鼠抗人 EGF 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Human EGF Conjugate	辣根过氧化物酶偶联 EGF 抗体	1 瓶
Human EGF Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Assay Diluent RD1-6	含有防腐剂的缓冲蛋白基质。可能含有沉淀物。用*于血清/血浆/唾液样本。*	1 瓶
Calibrator Diluent（5×）/RD5E	含防腐剂的浓缩的标准品稀释液（5×）。*用于细胞培养上清液/尿液样本。*	1 瓶
Calibrator Diluent RD6N	含有防腐剂的动物血清。*用于血清/血浆/唾液样本。*	2 瓶
Wash Buffer Concentrate（25×）	浓缩洗涤缓冲液（25×）	1 瓶
TMB Substrate	TMB ELISA 底物溶液	2 瓶
Stop Solution	终止液	1 瓶
Plate Covers	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="8">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="4">2-8℃储存，最多 30 天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB 底物溶液</td></tr>
<tr><td>标准品</td><td>每次检测使用新的标准品。使用后丢弃。</td></tr>
<tr><td>检测液 RD1-6</td><td rowspan="2">2-8℃储存，最多 30 天*</td></tr>
<tr><td>标准品稀释液 RD6N</td></tr>
<tr><td>标准品稀释液（5×）/RD5E</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的（1×）标准品稀释液，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>
</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

D. 注意事项

- 唾液中可检测到EGF。在进行本试验时，采取预防措施防止试剂盒试剂被污染。
- 本试剂盒中的某些成分含有ProClin，可能会引起皮肤过敏反应。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

下面列出的样本收集与储存条件仅为一般性指南。样本稳定性尚未经过评估。

细胞培养上清：离心去除颗粒物。样本可以立即分析，或分装后保存于≤-20℃。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RD6N稀释。

贫血小板血浆 - 使用EDTA、肝素或枸橼酸盐作为抗凝剂采集血浆。采集后30分钟内，以1000 × g离心15分钟。建议将血浆在2-8℃下以10,000 × g再次离心10分钟，以完全去除血小板。立即检测，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RD6N进行稀释。

EGF存在于血小板颗粒中，并在血小板活化时释放。因此，测定循环中的EGF水平时应采集贫血小板血浆。需注意，许多血浆制备方案（包括临床与实验室标准协会（CLSI）推荐的程序）均无法完全去除血液中的血小板。

唾液 - 将唾液收集于试管中，以10,000 × g再次离心5分钟。收集水相层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RD6N进行稀释。

尿液 - 无菌采集当日第一次晨尿（中段尿），直接排入无菌容器中。离心去除颗粒物。立即检测，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×）进行稀释。

B. 样本准备工作

血清样本建议至少需要稀释20倍。建议的20倍稀释方法：即25 µL血清+475 µL标准品稀释液RD6N。最佳稀释度应由最终用户确定。

唾液样本由于其内源性水平较高，至少需要稀释10倍。建议的10倍稀释方法：取50 µL样本+450 µL标准品稀释液RD6N。最佳稀释度应由最终用户确定。

尿液样本至少需要稀释200倍。建议的200倍稀释方法：先将25 µL样本加入225 µL标准品稀释液（1×）*中，完成第一步稀释；再取50 µL上述稀释后样本加入950 µL标准品稀释液（1×）*中，即可达到200倍稀释。最佳稀释度应由最终用户确定。

*参见“试剂配制”部分。

C. 检测前准备工作

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

注：*唾液中含有高浓度的EGF，为避免污染，实验时请带口罩、手套。*

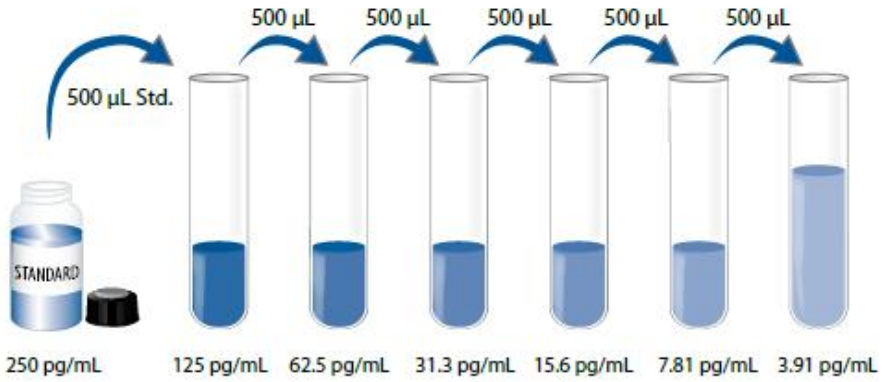
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

标准品稀释液（1×）：使用去离子水或蒸馏水稀释配制成标准品稀释液（1×）。将10 mL标准品稀释液（5×）/RD5E加入40 mL去离子水或蒸馏水中，以配制50 mL校准品稀释液（1×）。

人EGF标准品：开盖前请瞬时离心。冻干标准品的重溶体积请参考瓶身标签*。使用标准品稀释液（1×）（*用于细胞培养上清液/尿液样本*）或标准品稀释液RD6N（*用于血清/血浆/唾液样本*）复溶人EGF标准品。得到浓度为250 pg/mL标准品母液。轻轻震摇至少15分钟，其充分溶解。

*如有疑问，请咨询我们的技术支持。

向各个稀释管中加入500 µL标准品稀释液（1×）（*用于细胞培养上清液/尿液样本*）或标准品稀释液RD6N（*用于血清/血浆/唾液样本*）。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。250 pg/mL管作标准曲线最高点，相应的标准品稀释液（1×）/RD6N可用作标准曲线零点（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底物溶液上板顺序一致；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

注意：*唾液中存在EGF。建议使用口罩和手套来保护试剂盒免受污染。*

1. 按照上一节的说明，准备好所有需要的试剂、样品和标准品；
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. **仅适用于血清/血浆/唾液样本：**每孔加入50 µL检测液RD1-6。检测液RD1-6中可能含有沉淀物。*使用前及使用过程中应充分混匀。*
4. 分别将不同浓度标准品或者实验样本*加入相应孔中，每孔200 µL。用封板膜封住反应孔，**室温孵育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；
5. 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2次，共操作3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；
6. 在每个微孔内加入200 µL酶标检测抗体。用封板膜封住反应孔。

   适用于细胞培养上清液/尿液样本：室温孵育1小时；

   适用于血清/血浆/唾液样本：室温孵育2小时；
7. 重复第5步洗板操作；
8. 在每个微孔内加入200 µL TMB底物溶液，**室温孵育20分钟。注意避光；**
9. 在每个微孔内加入50 µL终止液，孔内溶液颜色会从蓝色变为黄色。如果溶液颜色变为绿色或者颜色变化不一致，请轻拍微孔板，使溶液混合均匀；
10. 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；；
11. **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EGF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。

    *样本可能需要稀释。参见“样本制备”部分。

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

