Quantikine® ELISA

Human EGF Immunoassay

Catalog Number DEG00
SEG00
PDEG00

For the quantitative determination of human Epidermal Growth Factor (EGF) concentrations in cell culture supernates, serum, platelet-poor plasma, saliva and urine.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

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).

The Quantikine Human EGF Immunoassay kit is a 3.5-4.5 hour solid phase ELISA designed to measure EGF levels in cell culture supernates, serum, platelet-poor plasma, saliva, and urine. It contains *E. coli*-derived recombinant human EGF and antibodies raised against the recombinant factor. It has been shown to accurately quantitate recombinant human EGF. Results obtained measuring natural human EGF showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that this kit can be used to determine relative mass values for natural human EGF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human EGF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any EGF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human EGF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of EGF bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- 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.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. 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 Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DEG00	CATALOG # SEG00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human EGF Microplate	890090	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human EGF.	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.*
Human EGF Standard	890092	2 vials	12 vials	Recombinant human EGF in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard after use. Use a new standard for each assay.
Human EGF Conjugate	890091	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human EGF conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-6	895158	1 vial	3 vials	11 mL/vial of a buffered protein base with preservatives. *May contain a precipitate. For serum/plasma/ saliva samples.*	
Calibrator Diluent RD5E Concentrate	895080	1 vial	6 vials	21 mL/vial of a 5-fold concentrated buffered protein base with preservatives. *For cell culture supernate/urine samples. Use diluted 1:5 in this assay.*	
Calibrator Diluent RD6N	895135	2 vials	12 vials	21 mL/vial of animal serum with preservatives. *For serum/plasma/ saliva samples.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DEG00 contains sufficient materials to run an ELISA on one 96 well plate.
SEG00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDEG00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

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.
- 100 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.
- Human EGF Controls (optional; R&D Systems, Catalog # QC21).

PRECAUTIONS

EGF is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

Assay Diluent RD1-6 contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

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SAMPLE COLLECTION & 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.

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.

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.

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.

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.

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.

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.

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 RD5E (diluted 1:5)*. Complete the 200-fold dilution by adding 50 µL of the diluted sample to 950 µL of Calibrator Diluent RD5E (diluted 1:5).

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

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

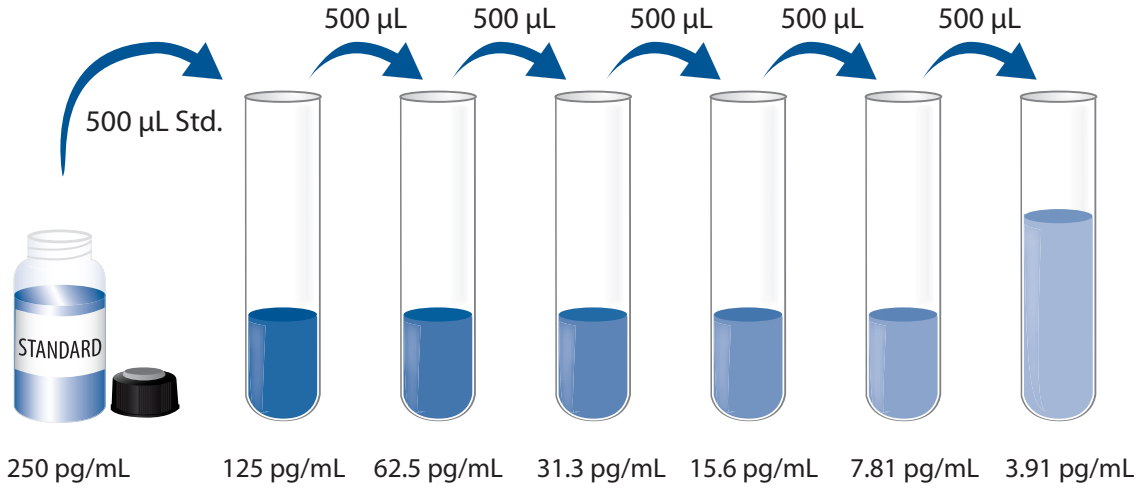
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5E (diluted 1:5) - Add 10 mL of Calibrator Diluent RD5E Concentrate to 40 mL of deionized or distilled water to yield 50 mL of Calibrator Diluent RD5E (diluted 1:5).

Human EGF Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human EGF Standard with Calibrator Diluent RD5E (diluted 1:5) (*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.

Pipette 500 µL of the appropriate Calibrator Diluent into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard serves as the high standard (250 pg/mL). The appropriate Calibrator Diluent serves as the zero standard (0 pg/mL).



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ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls 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, working standards, and samples 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, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process twice for a total of three 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.
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 Substrate Solution 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 the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. (O.D.)

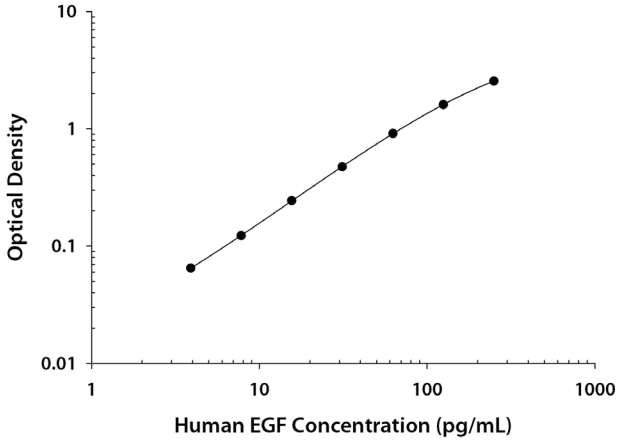
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human 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.

TYPICAL DATA

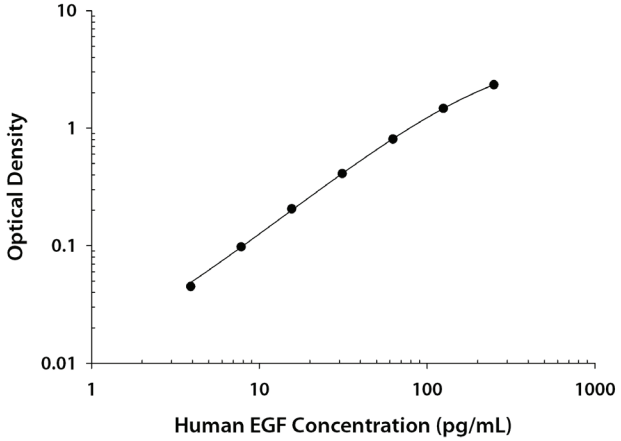
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

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

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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. Assays were performed by at least three technicians using two lots of components.

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

RECOVERY

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

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human EGF in various matrices and diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the 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

*Samples were diluted prior to assay.

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.

CALIBRATION

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

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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	4.54-5.20
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 leukocytes (PBL; 1 x 10^6 cells) were cultured in RPMI 1640 and supplemented with 10% fetal bovine serum. Cells were cultured 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.

SPECIFICITY

This assay recognizes natural and recombinant human EGF.

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

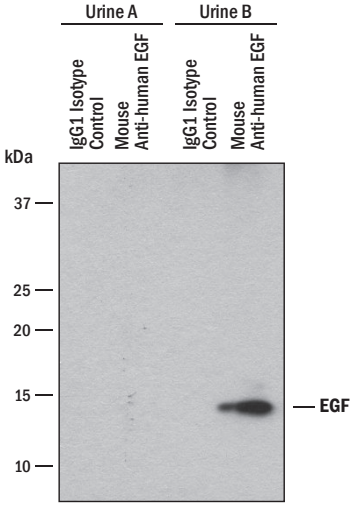
Recombinant human:

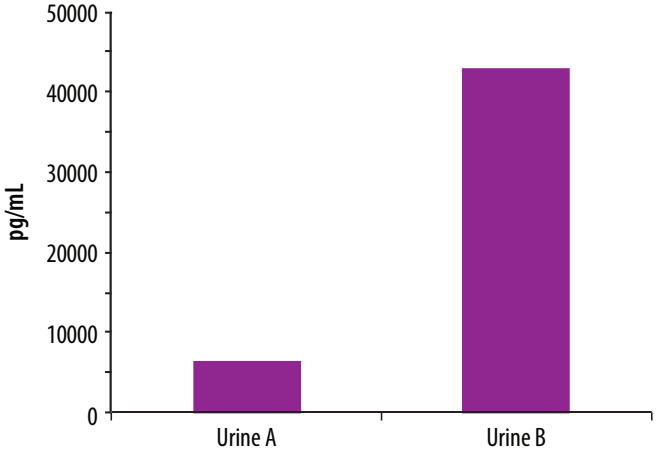
Amphiregulin
Betacellulin
Cripto-1
EGF R
Epigen
Epiregulin
ErbB2
ErbB3
ErbB4
HB-EGF
MFG-E8
NRG1/HRG1
NRG1 Isoform SMDF
NRG1-α/HRG1-α EGF Domain
NRG1-β1/HRG1-β1 EGF Domain
NRG1-β1/HRG1-β1 ECD
TGF-α
TNF-α

Recombinant mouse:

Amphiregulin
Betacellulin
Cripto
EGF
pro-EGF
EGF R
Epigen
Epiregulin
ErbB3
ErbB4
MFG-E8

Recombinant rat EGF cross-reacts approximately 1.0%, and recombinant human Pro-EGF cross-reacts approximately 1.3% in this assay.





Human urine samples were analyzed by Immunoprecipitation/Western blot and Quantikine 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.

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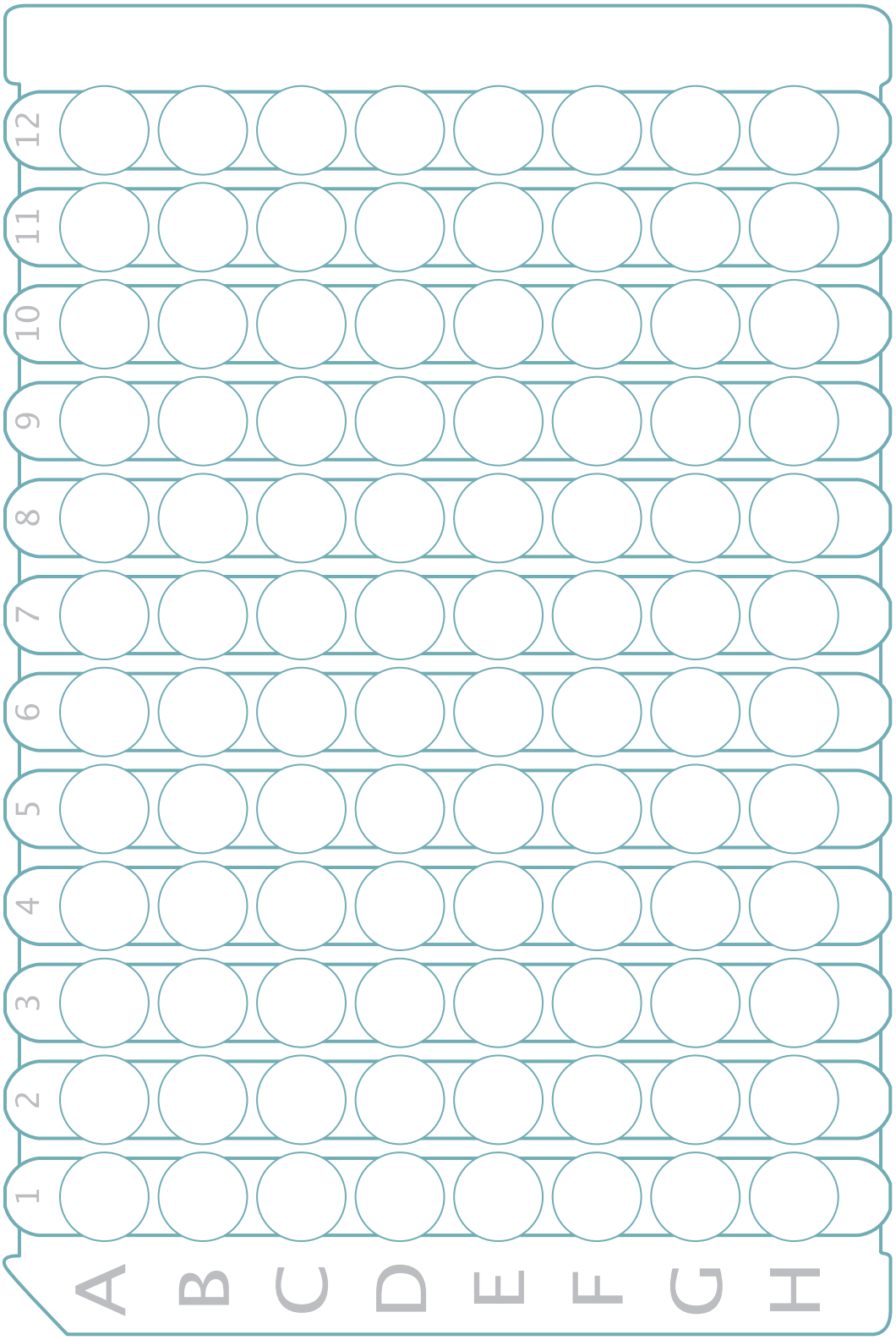
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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