Human EGF ELISA Kit

For the quantitative determination of human epidermal growth factor (EGF) concentrations in serum, plasma, cell culture supernatant, urine, and other biological fluids.

Catalogue Number: EL10010

96 Wells

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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S7.5 (03) EGF

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INTENDED USE

This Human EGF ELISA kit is to be used for the *in vitro* quantitative determination of human epidermal growth factor (EGF) concentrations in serum, plasma, cell culture supernatant, urine, and other biological fluids. This kit is intended FOR LABORATORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

INTRODUCTION

Epidermal growth factor (EGF), a polypeptide mitogen, was first observed in 1959 by Cohen and Levi-Montalcini while studying Nerve Growth Factor (NGF) in snake venom extracts.[1] It was subsequently isolated and purified from mouse submandibular glands. When injected into new-born mice this new factor caused precocious eyelid opening and incisor eruption.[2,3] EGF was further purified, based on its ability to induce the proliferation of basal skin cells.[4] Also, a potent inhibitor of gastric acid secretion was identified and isolated from the urine of a pregnant women, and named human ß-urogastrone. It was shown that this protein was very similar to purified human EGF.[5,6]

The cDNA cloning of mature EGF revealed that this polypeptide (6 kDa, 53 amino acids) belonged to a family of growth factors (TGF-α, vaccinia virus growth factor and amphiregulin) that bind to the same 160-185 kDa family of cell surface receptors. Structurally EGF is homologous to TGF-α.[7,8,9] Mature EGF is formed when the large precursor molecule (pre-EGF), a transmembrane protein (130 kDa, 1217 amino acids) which contains at least seven EGF-like sequences is cleaved. Within this precursor molecule, it is the sequence closest to the C-terminus of the mRNA that represents the coding region for the mature, soluble EGF sequence.[7,10] The uncleaved precursor shows biological activity and is capable of binding to the EGF receptor.[11,12] The physiological significance of the EGF precursor, nor the mechanisms of its processing into mature EGF have yet to be illustrated. However it is a source for soluble EGF and may be involved in cell to cell signalling or "juxacrine" action by negotiating intercellular communication between membrane-anchored EGF and cells with EGF receptors on their surfaces.[7,11] A soluble form of the EGF precursor molecule has been shown to be a heparin-binding protein, upon cleavage and release of the precursor extracellular domain.[12]

EGF is found in a variety of bodily fluids and tissues, but lower amounts are generally found in the latter. Substantial quantities of EGF are seen in saliva, bile, pancreatic juice, amniotic fluid, mammary fluids and secretions, gastric and duodenal contents, prostatic and seminal fluids, and urine.[7,11] Lower amounts of EGF can be found in whole blood and platelet poor plasma, when compared to other body fluids. EGF may be released from platelets during blood coagulation, since EGF levels are increased in serum, when compared to plasma.[13] EGF stimulates the growth of many tissues[3] and contributes to a wide variety of *in vitro* and *in vivo* biological effects, which are varied and depend on the target tissue. *In vivo*, EGF promotes angiogenesis, liver regeneration, epithelial development, acceleration of wound healing, and inhibits gastric acid secretion.[9] It has been shown that EGF *in vitro* promotes colony formation of epithelial cells in culture,[14] suppress insulin-induced glucokinase (hexokinase IV) activity,[15] promotes chemomigration of prostate tumor cell line[(16)] and is a mitogen for fibroblasts and endothelial cells.[17-18] Thus, EGF may play a role in human health and disease and could have potential therapeutic role in wound healing, regeneration of liver tissue and repair, embryonic and infant development, and prevention of vaccinia virus infection.[9]

This EGF ELISA is a 2.5 hour solid phase immunoassay readily applicable to measure EGF levels in serum, plasma, cell culture supernatant, urine, and other biological fluids in the range of 0 to 1000 pg/mL. It showed no cross reactivity with other cytokines tested. This assay recognizes natural human EGF, recombinant EGF, and high molecular weight forms of EGF in human urine. This EGF ELISA is expected to be effectively used for further investigations into the relationship between EGF and the various conditions mentioned.

PRINCIPLE OF THE ASSAY

This EGF enzyme-linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal specific for EGF. Standards or samples are then added to the appropriate microtiter plate wells and incubated. EGF, if present, will bind and become immobilized by the antibody pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove unbound EGF and other components of sample. In order to quantitate the amount of EGF present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated monoclonal antibody specific for EGF is added to each well to "sandwich" the EGF immobilized during the first incubation. The microtiter plate then undergoes a second incubation. The wells are thoroughly washed to remove all unbound HRP-conjugated antibodies and a TMB (3,3'5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain EGF and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

In order to measure the concentration of EGF in the samples, this kit contains two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant/ urine testing). According to the testing system, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D.) versus EGF concentration (pg/mL). The concentration of EGF in the samples is then determined by comparing the O.D. of the samples to the standard curve.

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

	96 tests

1. **EGF MICROTITER PLATE** (Part EL10-1) ***96 wells***
   Pre-coated with anti-human EGF monoclonal antibody.

2. **EGF CONJUGATE** (Part EL10-2) ***12 mL***
   Anti-human EGF monoclonal antibody conjugated to horseradish peroxidase with preservative.

3. **EGF STANDARD** (Part EL10-3) ***2 vials***
   Recombinant human EGF (2 ng/vial) in a buffered protein base with preservative, lyophilized.

4. **CALIBRATOR DILUENT I** (Part EL10-4) ***25 mL***
   Animal serum with preservative. *For serum/plasma testing.*

5. **CALIBRATOR DILUENT II** (Part EL10-5) ***25 mL***
   Cell culture medium with calf serum and preservative. *For cell culture supernatant/urine testing.*

6. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
   20-fold concentrated solution of buffered surfactant.

7. **SUBSTRATE A** (Part EL10-6) ***10 mL***
   Buffered solution with Urea H_2O_2.

8. **SUBSTRATE B** (Part 30007) ***10 mL***
   Buffered solution with TMB.

9. **STOP SOLUTION** (Part 30008) ***14 mL***
   2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100µL and 50-200µL for running the assay.
2. Pipettes: 1 mL, 5 mL 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm ± 2nm)
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
   Solid Wastes: Autoclave for 60 minutes at 121°C.
   Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the virus before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form, which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

1. COLLECTION, HANDLING, AND STORAGE

a) **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.
b) **Serum**: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.
c) **Plasma:** Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, after collection there must be quick separation of plasma with less than 30 minutes on ice. Centrifuge for 10 minutes (4°C) to remove any particulate.
d) **Urine:** Collect first urine of the day (mid-stream), aseptically into a sterile container. Centrifuge to remove any visible particulate material.

- Avoid hemolytic, lipidic or turbid samples.
- Samples should be used immediately. Otherwise, samples must be aliquoted and stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

2. DILUTION PROCEDURES

Urine samples: Require a final dilution of 1:200 in order to detect normal EGF levels. Dilute urine samples with Calibrator Diluent II. The suggested dilution procedure is as follows:

Step 1: 20 µL urine sample + 180 µL of Calibrator Diluent II. (1:10)
Step 2: Transfer 20 µL diluted urine sample from Step 1 + 380 µL of Calibrator Diluent II. (1:200)

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

3. **EGF Standard:**

a) Two vials of Standards are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute the EGF Standard with either 2.0 mL of Calibrator Diluent I (for serum/plasma testing) or Calibrator Diluent II (for cell culture supernatant/urine testing). This reconstitution produces a stock solution of 1000 pg/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. The EGF standard stock solution can be stored frozen (-20°C) for up to 30 days. Avoid freeze-thaw cycles; aliquot if repeated use is expected.

b) Use the above stock solution to produce a serial 2-fold dilution series within the range of this assay (15.6 pg/mL to 1000 pg/mL) as illustrated. Add 0.5 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted EGF Standard will serve as the high standard (1000 pg/mL) and the Calibrator Diluent will serve as the zero standard (0 pg/mL).

ASSAY PROCEDURE

1. Prepare Wash Buffer and EGF Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards or Samples to the Microtiter Plate.*

Wells	Contents	**Wells**	Contents
1A, 1B	Standard 1 - **0 pg/mL** (S1)	**2A, 2B**	Standard 5 –**125 g/mL** (S5)
1C, 1D	Standard 2 –**15.6 pg/mL** (S2)	**2C, 2D**	Standard 6 - **250 pg/mL** (S6)
1E, 1F	Standard 3 - **31.3 pg/mL** (S3)	**2E, 2F**	Standard 7 - **500 pg/mL** (S7)
1G, 1H	Standard 4 - **62.5 pg/mL** (S4)	**2G, 2H**	Standard 8 - **1000 pg/mL** (S8)
3A-12H	EGF samples		

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S5	1	5	9	13	17	21	25	29	33	37
B	S1	S5	1	5	9	13	17	21	25	29	33	37
C	S2	S6	2	6	10	14	18	22	26	30	34	38
D	S2	S6	2	6	10	14	18	22	26	30	34	38
E	S3	S7	3	7	11	15	19	23	27	31	35	39
F	S3	S7	3	7	11	15	19	23	27	31	35	39
G	S4	S8	4	8	12	16	20	24	28	32	36	40
H	S4	S8	4	8	12	16	20	24	28	32	36	40

2. Add 100 μL of Standard or sample to the appropriate well of the antibody pre-coated Microtiter Plate. Cover and incubate for 1 hour at room temperature.

3. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 μL/well/wash (range: 350-400 μL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

4. Dispense 100μL of conjugate to each well. Cover and incubate for 1 hour at room temperature.

5. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).

6. Repeat wash procedure as described in Step 3.

7. Add 100 μL Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.

8. Add 100 μL Stop Solution to each well. Mix, by gently tapping the plate.

9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader set within 30 minutes.

CALCULATION OF RESULTS

The standard curve is used to determine the amount of EGF in an unkown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding EGF concentration (pg/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 pg/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of EGF 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 EGF concentration. Urine samples have been 1:200 diluted, the concentration read from the standard curve must be multiplied by the dilution factor ($\times$200).

3. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

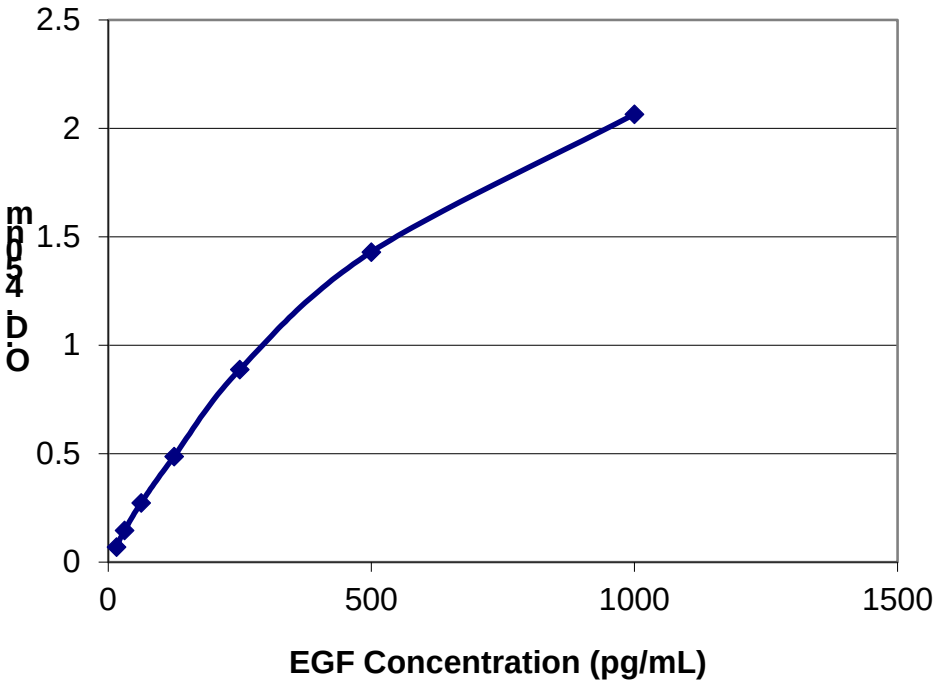
TYPICAL DATA

Results of a typical standard run of an EGF ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain their own standard curve

EXAMPLE ONE

The following data was obtained for a standard curve using Calibrator Diluent I.

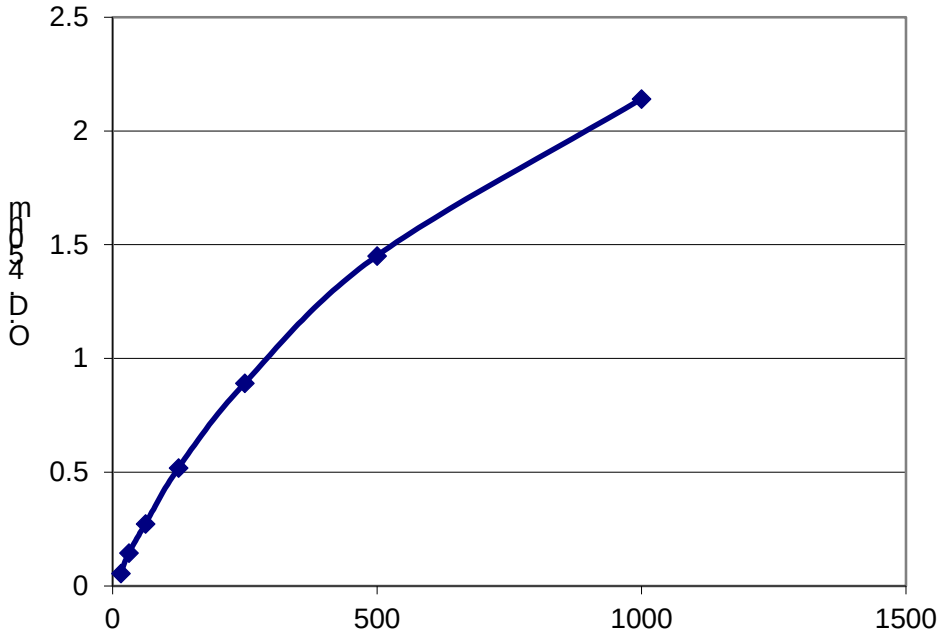
Standard (pg/mL)	Mean	Co-efficient of Variation	Zero Standard Subtracted (Std.) -(S1)
0	0.058	7.31	0
15.6	0.1270	0.00	0.0690
31.3	0.2030	1.39	0.1450
62.5	0.3300	0.43	0.2720
125	0.5445	0.39	0.4865
250	0.9435	0.67	0.8873
500	1.4870	1.05	1.4290
1000	2.1225	2.37	2.0645



EXAMPLE TWO

The following data was obtained for a standard curve using Calibrator Diluent II.

Standard (pg/mL)	Mean OD450	Co-efficient of Variation	Zero Standard Subtracted (Std.)-(S1)
0	0.0847	3.57	0
15.6	0.1397	2.89	0.0550
31.3	0.2093	4.06	0.1446
62.5	0.3373	2.38	0.2726
125	0.5833	4.42	0.5186
250	0.9550	3.54	0.8903
500	1.5153	1.66	1.4506
1000	2.2047	0.73	2.1401



PERFORMANCE CHARACTERISTICS

1. **INTRA-ASSAY PRECISION**
   To determine within-run precision, three different samples of known concentration were assayed by using 17-20 replicates in 1 assay.

	Calibrator Diluent II assay		
Sample	**1**	**2**	**3**
n	17	20	20
Mean (pg/mL)	102.6	311.1	1000.0
Standard Deviation (pg/mL)	5.7	8.1	33.0
Coefficient of Variation (%)	*5.6*	*2.6*	*3.3*

2. **INTER-ASSAY PRECISION**
   To determine between-run precision, three different samples of known concentration were assayed by using replicates on 20 different assays.

	Calibrator Diluent II assay		
Sample	**1**	**2**	**3**
n	20	20	20
Mean (pg/mL)	113.0	334.3	1000.0
Standard Deviation (pg/mL)	8.4	17.5	62.0

	Calibrator Diluent II assay		
Sample	**1**	**2**	**3**
Coefficient of Variation (%)	*7.4*	*5.2*	*6.2*

3. **RECOVERY**
The recovery of EGF spiked to 3 different levels throughout the range of the assay In various matrices was evaluated.

Sample Type	Average Range %	Range %
Cell culture media	96	85 -106
Serum	100	95 - 116
Plasma	102	88 - 114

4. **SENSITIVITY**
The minimum detectable dose of EGF was determined by adding two standard deviations to the mean optical density value of the 20 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose using a standard curve generated with Calibrator Diluent I is *20 pg/mL* and using Calibrator Diluent II is *15 pg/mL*.

5. **SPECIFICITY**
This sandwich ELISA recognises both natural and recombinant human EGF. This kit exhibits no detectable cross-reactivity with human; IL-8, IL-1ß, MCAF, TGF-α, TGF-ß, SAA, MCP-3, TNF-α, RANTES, EPO, M-CSF, GM-CSF, and FGF.

6. **CALIBRATION**
This immunoassay is calibrated against W.H.O First International Standard (1994 91/530).

7. **EXPECTED NORMAL VALUES**
Biological samples from apparently healthy, normal individuals were collected and the average EGF concentration measured. Serum samples (n=14) averaged 240 pg/mL. Plasma samples collected with EDTA, heparin, and citrate (n=14) were all below the lowest standard, 31.3 pg/mL. Urine samples (n=19) averaged 57 ng/mL.

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