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KIT DIRECTION BOOKLET
HUMAN EGF ELISA

Catalog Number: BT- 720

Introduction

Epidermal Growth Factor (EGF) is a 53 amino acid polypeptide, first isolated from the mouse submaxillary gland (1,12,13). The peptide is a potent stimulator of growth in numerous tissues of mouse and human (2,3). In man, human EGF (hEGF) has been detected in several tissues including the thyroid, pancreas, duodenum, submandibular gland and kidney (3) and also in plasma, saliva, milk and urine (4,5). It was recently established that blood platelets contain significant quantities of hEGF (7). Platelets may be the source of most if not all hEGF found in plasma; plasma hEGF concentrations are reportedly strongly dependent on blood processing techniques (8) and true plasma hEGF may be very low.

Although the physiological role for hEGF has not been established, its presence in platelets along with platelet-derived growth factor (PdGF) and transforming growth factor-beta (TGF-β), suggests a wound healing function for platelet hEGF.

Human EGF is identical to B-urogastrone (6), a potent inhibitor of gastric acid secretion. This property in addition to the presence of hEGF in milk and colostrum, the duodenum and pancreas, implicates hEGF in the biology of the gut. Epidermal growth factor may also have an important role in male reproduction. Removal of the submandibular gland of mature male mice, resulted in significant decrease of mature sperm in the epididymis and spermatids in the testes. This coincided with loss of plasma EGF but not plasma testosterone or follicle-stimulating hormone (9). Immune hEGF activity in seminal fluid has been reported (10). Epidermal growth factor is also potentially a marker for some tumors; hEGF was localized in tissue sections of lung and pancreatic cancers (11).

The BTI Human EGF ELISA Kit is offered as a convenient, versatile and accurate system to aid researchers in the quantitative analysis of hEGF in a variety of biological fluids and tissue extracts.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMAN OR DIAGNOSTIC PROCEDURES (REV. 12/02)

Description

The BTI Human EGF Elisa Kit employs a competitive protein binding technique in which a biotinylated hEGF competes with unlabeled hEGF for a limited number of specific antibody binding sites immobilized to the polystyrene wells. The percentage of antibody bound biotinylated-hEGF decreases as a function of increasing unlabeled hEGF. The biotin groups are then determined by incubation with a streptavidin-horseradish peroxidase and subsequent color development. Absorbance which is inversely proportional to hEGF concentration, is measured with a suitable spectrophotometer (a microplate reader). hEGF in samples is determined by comparison with a standard curve prepared with a series of hEGF samples of known concentration.

Sample Preparation

All samples not assayed immediately should be stored at -20◦C or lower. Cell culture, plasma and urine can be assayed directly. Avoid the use of azide since it inhibits the enzyme conjugate. See reference 7 before assaying plasma (blood platelets contain EGF).

Materials Required but not Supplied

1. A 96-well microtiter plate reader to measure absorbance at approx. 450nm.
2. Precision Micropipets.
3. A 37◦C Incubator.
4. An automated or manual 8-channel plate washer is recommended.
5. Deionized or distilled water.

Reagents: Description and Preparation (All components stable 10 months 4◦C).

1. Working sample buffer **BT-491**. One 125ml bottle. Store at 4◦C.

2. Phosphate-Saline Concentrate (Wash Buffer). **BT-492**. One 100ml Bottle. Dilute contents to 500ml with deionized water. Adjust pH to 7.5 if necessary. Store at 4◦C.

3. Human EGF Standard. **BT-451**. One Vial. 100ng lyophilized. Reconstitute with 1ml sample buffer to give a concentration of 100ng/ml. Store at 4◦C for 1 month or at -20◦C for 6 months. This is the stock for preparation of working standards.

4. Biotinylated-hEGF. **BT-724**. One Vial. Reconstitute with 6ml of sample buffer. The solution is stable at 4◦C for a minimum of 3 months.

5. Streptavidin-Horseradish Peroxidase. **BT-465.** One Vial. 11ml solution. Store at 4◦C.

6. Peroxidase Substrate. TMB (3,3',5,5'-Tetramethyl-benzidine). **BT-497.** One Vial. Store at 4◦C.

7. Hydrogen Peroxide. **BT-498**. One Vial. Store at 4◦C.

8. Stop Solution. **BT-499.** One Vial. Store at 4◦C.

9. One 96-well microtiter plate - 8 well strips. Coated with rabbit anti-human EGF.

10. Human Urine Control. **BT-729**. Lyophilized. Reconstitute the vial with 500ul diluent buffer.

Store at -20°C. The concentration of hEGF is printed on the label .

ASSAY PROCEDURE

1) Preparation of Working Standards.
Dilute the hEGF stock (100ng/ml, reagent 3, **BT-451)** to give a set of working standards in the range of 0.5 to 100ng/ml using the sample buffer **(BT-491).** One dilution scheme follows:

Concentration (ng/ml)	Description	Tube I.D.
100.0	Stock	S-1
25.0	100ul S-1 + 300ul buffer	S-2
5.0	100ul S-2 + 400ul buffer	S-3
2.5	100ul S-3 + 100ul buffer	S-4
1.0	100ul S-3 + 400ul buffer	S-5
0.5	100ul S-5 + 100ul buffer	S-6

Have all other samples prepared before pipeting solutions into wells.

2) If not using the entire plate at once, remove strips not in use immediately and store them dry at 4°C.

Do all determinations in duplicate, using 2 wells maximum binding, Bmax, 2 wells each for the standards and unknowns and controls.

3) Pipet 50ul diluent buffer in the Bmax wells and 50ul of standards, controls and unknowns into appropriate wells.

4) Pipet 50ul of the biotinylated-hEGF solution (**BT-724**) into all wells. Mix gently about 1 minute. Cover wells tightly with parafilm or sealing tape, incubate at 37°C, 2½ hours.

The pipeting steps 3 and 4 should be completed in about 20 minutes.

5) Aspirate all wells, fill each with approximately 300ul wash buffer and aspirate. Repeat the wash 2 times. Gently tap the plate on paper towels to remove excess liquid.

6) Pipet 100ul of the Streptavidin-peroxidase (**BT-465**) in all wells, mix gently, incubate at room temperature, 30 minutes.

7) Mix the TMB (**BT-497**) and hydrogen peroxide (**BT-498**) solutions in equal volumes (these should have been equilibrated at room temperature and mix only an amount sufficient for the number of wells in use). Wash the wells as in step 5 and immediately add 100ul of the above substrate to all wells. Incubate at room temperature, in the dark, 10 minutes.

8) Add 100ul stop solution (**BT-499**). Mix and measure absorbance at 450nm (make sure there are no air bubbles in the solution and that the bottom of each well is clean and dry).

Calculations

Average duplicates. Divide the value of each standard, control and unknown by the Bmax value and multiply by 100. Obtain a standard curve plotting the %Bmax vs. ng/ml of each standard. Determine concentrations of controls and unknowns using this standard curve.

Specifications

Sample size: 50 ul
Assay time: 3.5 hrs
Sensitivity: 0.5 ng/ml
Range: 0.5 - 100 ng/ml
Intra-assay variation: 5%
Inter-assay variation: 7%
Expected Range in normal urine: 5 - 50 ng/ml

References

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Typical Standard Curve