DRG® Human EGF (EIA-1734)

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Please use only the valid version of the package insert provided with the kit.

Introduction

The 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

Description

The 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. 450 nm.
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 at 4°C).

1. **Working sample buffer**.
   One 125 ml bottle. Store at 4°C.

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

3. **Human EGF Standard**.
   One vial. 100 ng lyophilized.
   Reconstitute with 1 ml sample buffer to give a concentration of 100 ng/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**.
   One vial.
   Reconstitute with 12ml of sample buffer.
   The solution is stable at 4°C for a minimum of 3 months.

5. **Streptavidin-Horseradish Peroxidase**.
   One vial. 11ml solution. Store at 4°C.

6. **Peroxidase Substrate**. TMB (3,3',5,5'-Tetramethyl-benzidine).
   One vial. Store at 4°C.

7. **Hydrogen Peroxide**.
   One vial. Store at 4°C.

8. **Stop Solution**.
   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**.
    Lyophilized.
    Reconstitute the vial with 500 µl sample 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 (100 ng/ml, reagent 3), to give a set of working standards in the range of 0.5 to 100 ng/ml using the sample buffer. One dilution scheme follows:

Concentration (ng/ml)	Description	Tube I.D.
100.0	Stock	S-1
25.0	100 µl S-1 + 300 µl buffer	S-2
5.0	100 µl S-2 + 400 µl buffer	S-3
2.5	100 µl S-3 + 100 µl buffer	S-4
1.0	100 µl S-3 + 400 µl buffer	S-5
0.5	100 µl S-5 + 100 µl 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 50 µl sample buffer in the Bmax wells and 50 µl of standards, controls and unknowns into appropriate wells.

4. Pipet 50 µl of the biotinylated-hEGF solution 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 300 µl wash buffer and aspirate. Repeat the wash 2 times.
   Gently tap the plate on paper towels to remove excess liquid.

6. Pipet 100 µl of the Streptavidin-peroxidase in all wells, mix gently,
   incubate at room temperature, 30 minutes.

7. Mix the TMB and hydrogen peroxide 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 100 µl of the above substrate to all wells.
   Incubate at room temperature, in the dark, 10 minutes.

8. Add 100 µl stop solution. 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.

References / Literature

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Typical Standard Curve