Human Estradiol E2 ELISA Kit

Catalog Number KAQ0622 (96 tests)

Pub. No. MAN0030245 Rev. A.0 (30)

Note: For safety and biohazard guidelines, see the "Safety" appendix in the following product documentation: *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Human Estradiol E2 ELISA Kit is a solid-phase competitive Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of 17-beta-estradiol (E2) in serum and plasma. The assay will recognize both natural and recombinant human estradiol E2.

17-beta-estradiol (E2) is a C-18 steroid hormone (molecular weight 272.4) produced mainly by the ovary and placenta, and in small amounts by adrenals and testes. Estradiol is in equilibrium with estrone, which can be converted to estriol by the liver and placenta.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KAQ0622 (96 tests)	Color code
Standard 0 pg/mL in human serum; contains preservatives. Refer to vial label for quantity and reconstitution volume	1 vial	Yellow
Controls 1 and 2 in human serum; contains preservatives. Refer to vial label for range	2 vials	Silver
Standards 1–6 in human serum; contains preservatives, ready-to-use, 1 mL/vial. Refer to vial label for concentration	6 vials	Yellow
Anti-Rabbit IgG-Coated Wells, 96-well strip-well plate	1 plate	Blue
Wash Buffer Concentrate (40X), 30 mL	1 bottle	Brown
Substrate Solution, Tetramethylbenzidine (TMB), ready-to-use	14 mL	White
Stop Solution; 0.5 M H_2SO_4	14 mL	Black

Materials required but not provided

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at thermofisher.com.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

Note: To avoid washerhead obstructions, preparing fresh diluted 1X Wash Buffer for assays is recommended.

- Dilute 30 mL of Wash Buffer Concentrate (40X) with 1,170 mL to a final volume of 1,200 mL. Label as 1X Wash Buffer.

  Note: The Wash Solution Concentrate is stable at room temperature until the expiration date.

Sample preparation guidelines

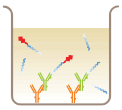
- Refer to the *ELISA Technical Guide* at thermofisher.com for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Store samples up to 7 days at 2–8°C prior to testing. Freeze samples at –20°C after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Perform Assay (Total assay time: 2 hours)

IMPORTANT! Perform a standard curve with each assay.

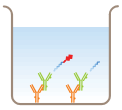
- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen



1. Add 25 µL of standards, controls, or samples to the appropriate wells.
2. Add 100 µL of 1X Estradiol-HRP Conjugate solution into each well except the chromogen blanks.
3. Incubate for 90 minutes at room temperature.
4. Thoroughly aspirate the solution and wash wells 3 times with 1X Wash Buffer.

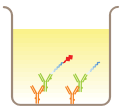
2 Add substrate solution



1. Add 100 µL of Substrate Solution to each well. The Substrate Solution begins to turn blue.
2. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

3 Add stop solution



Add 50 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance of each microwell on a spectrophotometer using 450 nm as the primary wavelength. You can optionally use 620 nm as the reference wave length, but 610 nm to 650 nm is acceptable. Blank the plate reader according to the manufacturer's instructions by using the blank wells. Optimally, the wells need to be read within 10 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve.

Performance characteristics

Standard curve example

The following data were obtained for the various standards over the range of 0 to 2,000 pg/mL human estradiol E2.

Standard Human Estradiol E2 (pg/mL)	Optical Density (450 nm)
2,000	0.28
1,000	0.55
500	0.85
250	1.15
100	1.49
25	1.83
0	2.09

Inter-assay precision

Samples were assayed 40 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	151.3	336.7	661.4
% Coefficient of Variation	14.9	10.8	6.9

Intra-assay precision

Samples of known human estradiol E2 concentrations were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	92.5	144.4	340.7
% Coefficient of Variation	9.2	9.0	8.7

Cross-reactivity

Substances	Cross-reactivity %	Substances	Cross-reactivity %
Estradiol 17-β	100	11-Deoxycortisol	0
Androstenedione	0	21-Deoxycortisol	0
Androsterone	0	Dihydrotestosterone	0
Corticosterone	0	Dihydroepiandrosterone	0
Cortisone	0	20-Dihydroprogesterone	0
Epiandrosterone	0	11-Hydroxyprogesterone	0
16-Epiestriol	0	17α-Hydroxyprogesterone	0.003
Estradiol-3-sulfate	0	17α-Pregnenolone	0
Estradiol-3-glucoronide	0	17α-Progesterone	0
Estradiol-17α	0	Pregnanediol	0
Estriol	2.27	Pregnanetriol	0
Estriol-16-glucoronide	0	Pregnenolone	0
Estrone	6.86	Progesterone	0
Estrone-3-sulfate	0	Testosterone	0.033
Dehydroepiandrosterone	0	Fulvestrant	3.7

Sensitivity

The minimum detectable dose of human estradiol E2 is 10.6 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Specificity

The percentage of cross-reaction was estimated under physiological conditions in serum by comparison of the concentration yielding a 50% binding inhibition.

Limited product warranty

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

The information in this guide is subject to change without notice.