

Newly Updated Estradiol ELISA Kit



A critical shortage of the antibody used in our Estradiol ELISA Kit (Item No. 582251) prompted the need for a replacement Estradiol ELISA Kit (Item No. 501890). Our scientists developed a new antiserum with less cross reactivity compared to the previous kit and changed the sample preparation/extraction protocol to a simpler and less hazardous format that provides improved specificity. Synthetic 17 β -estradiol and samples of human plasma were tested side by side in both kits to compare performance.

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NEW: Estradiol ELISA Kit

Item No. 501890

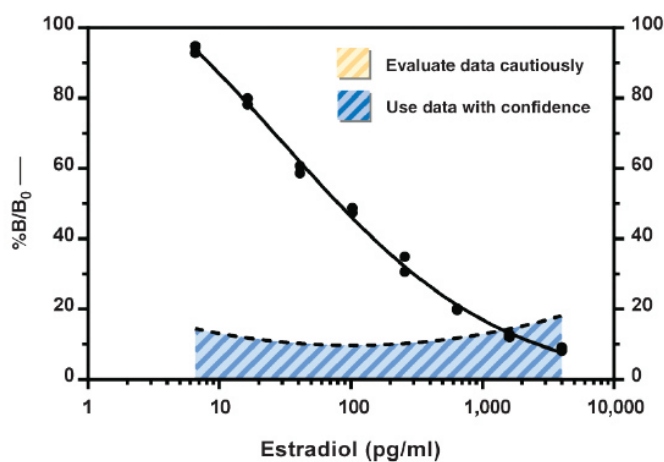
- Measure estradiol in plasma and serum samples
 - Assay 24 samples in triplicate or 36 samples in duplicate
 - Measure estradiol levels down to 20 pg/ml
 - **Incubation:** 2 hours | **Development:** 60 minutes
- Read:** Colorimetric at 414 nm



Sensitivity

The new kit offers sensitivity values comparable to those of the previous kit. However, the concentrations of analyte or assay values of the new kit are detectable over a wider assay range compared to the previous kit.

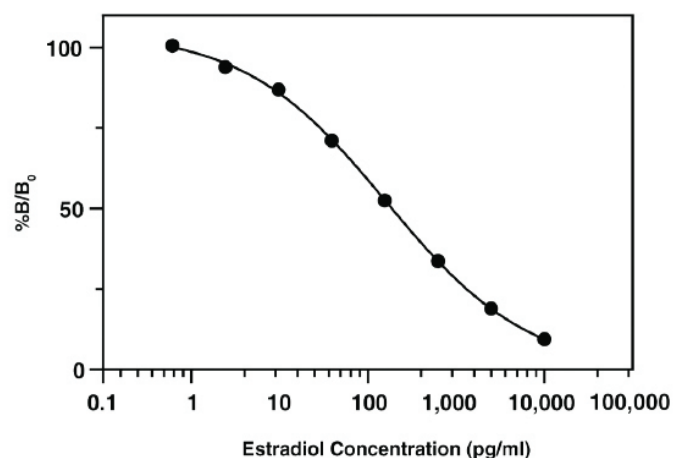
Previous Kit (Item No. 582251)



Assay Range = 6.6-4,000 pg/ml
Sensitivity (defined as 80% B/B₀) = 15 pg/ml
Mid-point (defined as 50% B/B₀) = 40-100 pg/ml

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted with ELISA Buffer.

New Kit (Item No. 501890)



Assay Range = 0.61-10,000 pg/ml
Sensitivity (defined as 80% B/B₀) = 20 pg/ml
Mid-point (defined as 50% B/B₀) = 188 pg/ml
Lower Limit of Detection (LLOD) = 6 pg/ml

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted with ELISA Buffer (1X).

Sample Preparation/Extraction Protocol

Plasma and serum samples may require extraction prior to quantification in the assay to eliminate potential substances like bulk proteins or lipids that may interfere with steroid quantification. Extraction can also help concentrate the sample to within the measurement range of the assay. We updated the extraction protocol in the new kit. This new protocol requires a single precipitation step with methanol, instead of the repeated liquid/liquid extraction steps needed with methylene chloride, which carries greater hazardous materials precautions.

Previous Kit (Item No. 582251) Protocol

- Methylene chloride used at 4X sample volume
- Extraction procedure is repeated 2 times

New Kit (Item No. 501890) Protocol

- Methanol used at 4X sample volume
- Precipitation procedure is one step

Cross Reactivity

The new kit demonstrates improved specificity for estradiol with minimal detection of estradiol 3-glucuronide, estrone, estradiol 3-sulfate, and estradiol 17-glucuronide.

| Compound | Previous Kit (Item No. 582251) | New Kit (Item No. 501890) |
|--------------------------------|--------------------------------|---------------------------|
| Estradiol | 100% | 100% |
| Methoxyestradiol | not tested | 2.5% |
| Estradiol 3-(β-D-Glucuronide) | 14% | 2.3% |
| Estrone | 12% | 1.38% |
| 2-Hydroxyestradiol | not tested | 1.3% |
| Estriol | 0.30% | 1.0% |
| Estradiol Benzoate | <0.01% | 0.7% |
| Estradiol 3-sulfate | 14.5% | 0.53% |
| Ethynyl Estradiol | 0.05% | 0.14% |
| 5-Androstan-17β-ol-3-one | 0.02% | 0.06% |
| 17α-Estradiol | <0.01% | 0.04% |
| 5α-dihydro Testosterone | 0.06% | 0.04% |
| Androstenediol | 0.02% | 0.03% |
| Testosterone | <0.01% | 0.03% |
| Estradiol 17-sulfate | <0.01% | 0.02% |
| Estradiol 17-(β-D-Glucuronide) | 10% | 0.02% |

For additional information on the performance of this kit, including spike and recovery and linearity data, please refer to the **kit booklet**.

Our technical support team can be reached at (734) 975-3888 or techserv@caymanchem.com to help answer any further questions about this replacement.

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