

Technical Bulletin

Changes in format for ET-1 EIA Kit

Catalog Number: 900-020 / 900-020A

Effective Date: October 30, 2007

Please note that as of the effective date above the antibodies, standard, and format of the ET-1 EIA Kit (Cat. No. 900-020) will be changed and the product given a new catalog number (Cat. No. 900-020A). The assay now consists of two monoclonal antibodies as opposed to two polyclonal antibodies, reducing assay time from overnight to 2 hours. The new assay format also requires less plate washing and offers more convenient incubation temperatures. In addition, standards have been recalibrated and reformatted from lyophilized to liquid. We recommend that any internal controls be reevaluated. Extensive validation studies have been completed, which are summarized below. The previous version of the kit will be available until March 2008 or while supplies last.

Assay Procedure Comparison

| Old Format (900-020) ET-1 EIA Kit | New Format (900-020A) ET-1 EIA Kit |
|---|---|
| Add 100 µL standard & sample to the wells | Add 100 µL standard & sample to the wells |
| Incubate overnight at 4°C | Incubate for 1 hour at RT |
| Wash 7 times | Wash 5 times |
| Add 100 µL of antibody to the wells | Add 100 µL of antibody to the wells |
| Incubate for 30 minutes at 37°C | Incubate for 30 minutes at RT |
| Wash 9 times | Wash 5 times |
| Add 100 µL of substrate to the wells | Add 100 µL of substrate to the wells |
| Incubate for 30 minutes at RT in the dark | Incubate for 30 minutes at RT |
| Add 100 µL stop solution | Add 100 µL stop solution |
| Read plate at 450 nm | Read plate at 450 nm |

Assay Specification Comparison

| Parameter | Old Format (900-020) | New Format (900-020A) |
|------------------------|--|---|
| Standard Range | 0.78 - 100 pg/mL | 0.78 - 100 pg/mL |
| Sensitivity | 0.14 pg/mL | 0.40 pg/mL |
| Time to Answer | Overnight | 2 hours |
| Incubation Conditions | 4°C, 37°C, and RT | RT |
| Standard | Lyophilized | Liquid |
| Storage | 4°C | 4°C |
| Capture Antibody | Polyclonal | Monoclonal |
| Detection Antibody | Polyclonal | Monoclonal |
| Sample Type & Dilution | Culture supernates (>1:2), serum (>1:32), and plasma (>1:8) | Culture supernates + 10% FBS (no dilution), serum (1:4), and plasma (1:4) |
| Cross Reactivity | ET-1 100%... ET-2, ET-3, Big ET-1, Big ET-2, Big ET-3 < 0.1% | ET-1 100%; ET-2 21%; ET-3 3.6 %, ... Big ET-1, Big ET-2, Big ET-3 < 0.1% |

In normal human plasma ET-2 is reported to be < 20% ET-1, Big ET-1 is 2x ET-1, and ET-3 is 50% ET-1.

Linearity of Dilution

Conditioned culture supernates containing 10% fetal bovine serum was serially diluted in the kit assay buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

| Dilution | Cell Culture Supernatant | | |
|----------|--------------------------|------------------|------------|
| | Measured (pg/mL) | Expected (pg/mL) | % Expected |
| 1 | 86.027 | --- | --- |
| 2 | 49.774 | 43.0135 | 115.7% |
| 4 | 24.177 | 21.5068 | 112.4% |
| 8 | 11.667 | 10.7534 | 108.5% |
| 16 | 6.192 | 5.37669 | 115.2% |
| 32 | 2.896 | 2.68834 | 107.7% |
| 64 | 1.411 | 1.34417 | 105.0% |

Comparison of Formats

The ET-1 standard in the kit was reformulated to more accurately reflect native ET-1 concentrations. The reformulated standard was quantified in both formats. The results, shown below indicate a factor of 1.33 should be applied to samples run in the revised kit (i.e., Old format result/ 1.33 = New format result).

