

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).

