

## **HtrA3 isoform specific ELISAs for early detection of preeclampsia**

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## Supplementary Material

**Supplemental Table 1. Characteristics of HtrA3 monoclonal antibody (mAb)**

HtrA3 mAb name	Detection of HtrA3 isoforms	
	HtrA3-L	HtrA3-S
2C4	+	+
9C9	+	+
10H10	+	+
3E6	+	-
6G6	+	-

**Supplemental Table 2. Linearity test for the newly established HtrA3 ELISAs**

Conditioned media (CM) from HEK293 cells transfected for the HtrA3-L or HtrA3-S isoform, and a pooled serum sample of first trimester pregnancy, were assayed in different dilutions and the percentage of recovery was calculated.

**HtrA3-T ELISA**

Sample	Dilution	Observed concentration (pM)	Expected concentration (pM)	Recovery
HtrA3-L CM	1/2	5683	5683	100%
	1/4	5255		92%
	1/8	4702		83%
HtrA3-S CM	1/2	3763	3763	100%
	1/4	4188		111%
	1/8	4419		117%
Serum	1/10	11329	11329	100%
	1/20	12490		110%
	1/30	13168		116%

**HtrA3-L ELISA**

Sample	Dilution	Observed concentration (pM)	Expected concentration (pM)	Recovery
HtrA3-L CM	1/2	3520	3520	100%
	1/4	3212		91%
	1/8	2768		79%
HtrA3-S CM	1/2	0	0	
	1/4	0		
	1/8	0		
Serum	1/5	3465	3465	100%
	1/10	3762		109%
	1/15	4518		130%

### **Supplemental Table 3. Spike and recovery test for the two HtrA3 ELISAs**

Recombinant HtrA3-L protein was spiked in an HtrA3-negative human serum sample and assayed by the HtrA3 ELISAs.

#### **HtrA3-T ELISA**

Spike level	Observed concentration (pM)	Expected concentration (pM)	Recovery
High	1499	1500	100%
Medium	752	750	100%
Low	370	375	99%

#### **HtrA3-L ELISA**

Spike level	Observed concentration (pM)	Expected concentration (pM)	Recovery
High	1500	1500	100%
Medium	750	750	100%
Low	373	375	100%

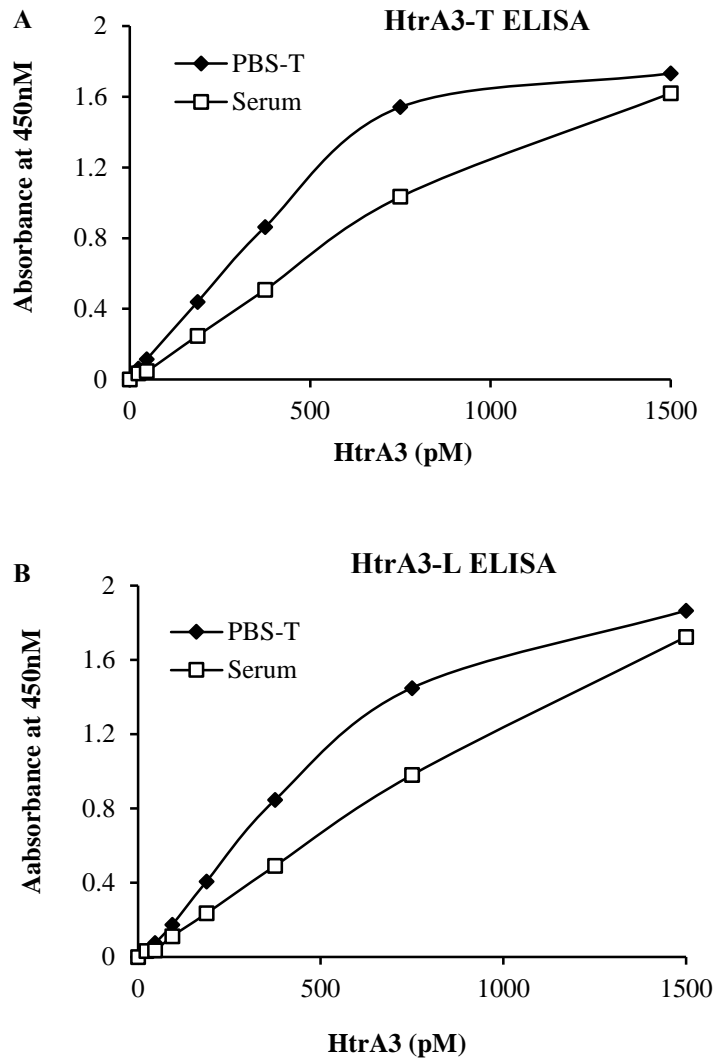
**Supplemental Table 4. Intra- and Inter-assay variations of the two HtrA3 ELISAs on standards and quality controls for serum detection**

**HtrA3-T ELISA**

Nominal HtrA3 concentration (pM)	Intra-assay %CV	Inter-assay %CV
1500	12%	5%
750	4%	4%
375	4%	3%
187.5	7%	4%
93.76	8%	6%
46.88	13%	67%
Quality controls		
High	2%	10%
Medium	4%	22%
Low	4%	17%

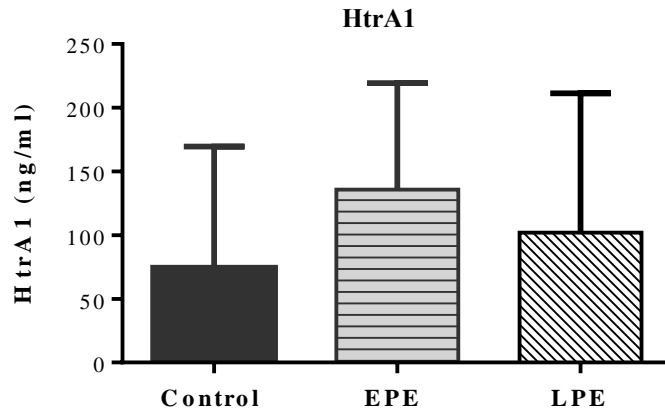
**HtrA3-L ELISA**

Nominal HtrA3 concentration (pM)	Intra-assay %CV	Inter-assay %CV
1500	2%	0%
750	3%	0%
375	5%	1%
187.5	5%	3%
93.76	6%	5%
46.88	12%	19%
23.44	14%	82%
Quality controls		
High	3%	7%
Medium	3%	6%
Low	4%	9%

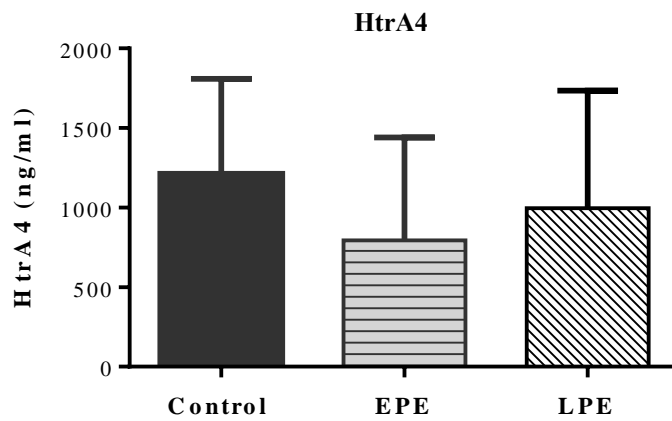


**Supplemental Figure 1. Representative standard curves of HtrA3 ELISAs in buffer and serum.** (A) HtrA3-T ELISA, (B) HtrA3-L ELISA. Serum matrix dampened the signals and modified the characteristics of the standards in both HtrA3 ELISAs.

**A**



**B**



**Supplemental Figure 2. Serum levels of HtrA1 and HtrA4 at 11-13 weeks of gestation in women who proceeded to term without complication or developed PE in the third trimester. (A) HtrA1. (B) HtrA4. Identical samples assayed for HtrA3 (Figure 3) were analyzed for HtrA1 and HtrA4 respectively. Control, n=292; early-onset PE (EPE), n=5; late-onset PE (LPE), n=36. Data are expressed as median  $\pm$  interquartile range.**