



**Tulsi Immuno Nano Research and Development LLP**

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LLPIN: AAT-8744, STARTUP CERTIFICATE NO: DIPP77265, UDYAM REGISTRATION NUMBER: UDYAM-HR-20-0004476

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## CERTIFICATE OF ANALYSIS

**Product Name:** Ready to use TMB substrate solution for HRP based ELISA

**Manufacturing Date:** XXXXXX

**Expiry Date:** XXXXXX

**The substrate is analysed for background and activity using following procedures.**

### **Background**

One hundred  $\mu\text{L}$  of TMB solution added in six wells of micro titre plate. Then 100  $\mu\text{L}$  of 0.5M sulphuric acid is pipetted in each well and, the absorbance is read at 450 nm against 620 nm reference filter. The average optical density of the six wells is calculated.

### **Activity**

Five  $\mu\text{L}$  of Medroxy-progesterone acetate-HRP conjugate at five dilutions is added in three wells each. 100  $\mu\text{L}$  of TMB solution is pipetted in each well and incubated for 10 minutes at room temperatures (18-25°C). The reaction is stopped by addition of 100  $\mu\text{L}$  of 0.5M sulphuric acid. The absorbance is read at 450 nm against 620 nm reference filter. The average optical density of the triplicate wells is calculated. The activity is compared either to that of previous batches of TMB solution or standard TMB solution of known activity.

### **Specifications**

The average background signal must be less than 0.05.

As activity is compared either to that of previous batches of TMB solution or standard TMB solution of known activity, the variation in activity should be below 15%.

**Appearance:** XXXX

**Background Signal:** XXXXX

**Activity test:** XXXXX

### **Storage**

Short term storage: room temperature, avoid direct contact with sunlight or UV light sources.

Long term storage: + 2-8°C, avoid direct contact with light and metal.

**Performed by QA**

XXXXXXXXXX

**Signature with date**