# Product Information

## STAT-8-Isoprostane EIA Standard

*Item No. 400434*

**IMPORTANT**

Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. UltraPure water may also be purchased (Item No. 400000).

**Laboratory Procedures**

The STAT-8-Isoprostane (STAT-8-*iso* $PGF_{2\alpha}$) EIA Standard you have purchased contains approximately 500 µl of 8-isoprostane as a solution in ethanol. The concentration of this standard is 300 ng/ml. For long term storage, we suggest that the STAT-8-isoprostane EIA standard be stored as supplied at -20°C; it will be stable for at least one year. When ready to use, equilibrate a pipette tip in ethanol by repeatedly filling and expelling the tip with ethanol several times. Using the equilibrated pipette tip, transfer 100 µl of the 8-isoprostane EIA standard into a clean test tube, then dilute with 900 µl UltraPure water. The concentration of this solution (the bulk standard) will be 30 ng/ml. Store this solution at 4°C.

To prepare the standard for use in EIA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 900 µl Tris Buffer to tube #1 and 500 µl Tris Buffer to tubes #2-8. Transfer 100 µl of the bulk standard (30 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 500 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 500 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. The concentrations of theses standards are 3,000, 1,500, 750, 375, 187.5, 93.8, 46.9, and 23.4 pg/ml. We recommend that you store these diluted standards for no more than 24 hours.

**Buffer Preparation**

1. **Tris Buffer**
   Prepare a 0.1 M Tris-HCl buffer, pH 7.4, containing 0.15 M sodium chloride, 2 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 0.05% azide, and 0.5% BSA solution by combining 12.11 g Tris base, 8.47 g sodium chloride, 0.406 g $MgCl_2 \cdot 6H_20$, 0.014 g $ZnCl_2$, 0.5 g sodium azide, and 5 g BSA in 800 ml UltraPure water and adjusting the pH to 7.4 with concentrated HCl. Stir at room temperature until completely dissolved and dilute to a total volume of 1.0 liter with UltraPure water. This buffer may also be purchased as a 10X concentrate buffer (Item No. 400080).
2. **Wash Buffer**
   Prepare a 0.01 M Tris-HCl buffer, pH 7.4, containing 0.05% Tween-20 by combining 1.3 g Tris-HCl and 0.194 g Tris base in 600 ml UltraPure water. Stir until dissolved and adjust the pH to 7.4 with HCl. Add 0.5 ml Tween 20 and dilute to a final volume of 1.0 liter with UltraPure water. This buffer may also be purchased as a 150X concentrated buffer (Item No. 411007).
3. **DEA Buffer**
   DEA Buffer may be purchased as a 10X concentrated buffer (Item No. 400082).



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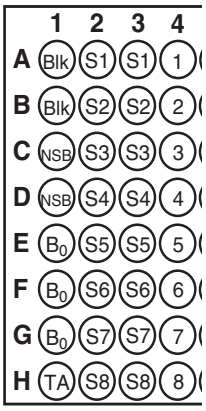
**Suggested Assay Protocol**

This standard has been tested and formulated to work exclusively with AP reagents. It is highly recommended that Cayman plates with Item No. 400004 or 400006 be used for this assay. This standard may not perform as described if used with other assay reagents or protocols. *NOTE: This is an abbreviated protocol. If you are not familiar with this assay, please contact us for a complete protocol.*

1. Add 100 µl of Tris Buffer to NSB wells and 50 µl to $B_0$ wells.
2. Add 50 µl of Standard or sample to the appropriate wells.
3. Add 50 µl Tracer (Item No. 400430) to all wells except Blk and TA.
4. Add 50 µl Antiserum (Item No. 400432) to all wells except Blk, TA, and NSB.
5. Incubate one hour at room temperature on an orbital shaker.
6. Wash the plate five times with Wash Buffer.
7. Add 200 µl *p*NPP to each well.
8. Add 5 µl Tracer to the TA well.
9. Develop for approximately 60-90 minutes ($B_0$ = 0.3-1.0 AU) on an orbital shaker.
10. Read absorbance at a wavelength between 405 and 420 nm.




**Blk**-Blank; **NSB**-Non-specific Binding; **$B_0$**-Maximum Binding; **TA**-Total Activity; **S1-S8**-Standards; **1-8**-Samples

**Related Product**

STAT-8-Isoprostane EIA Kit - Item No. 500431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