# 8-iso-Prostaglandin $F_{2\alpha}$ (direct) ELISA Kit

**Catalog Number: EKS-210**

**TOLL FREE 1-800-661-4978 FAX 250-744-2877**
**www.stressgen.com/reagents**

Table of Contents

| | |
|---|---|
| Assay Design | Page 2 |
| Scientific Overview | 2 |
| Precautions | 2 |
| Materials Provided | 3 |
| Storage of Materials | 3 |
| Materials Required but Not Provided | 3 |
| Critical Assay Parameters and Notes | 4 |
| Sample Preparation | 4 |
| Reagent Preparation | 5 |
| Assay Procedure | 6 |
| Calculation of Results | 7 |
| Typical Results | 7 |
| Typical Standard Curve | 8 |
| Typical Quality Control Parameters | 8 |
| Performance Characteristics | 9 |
| Sample Dilution Recommendations | 10 |
| References | 11 |

**FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.**

## Assay Design

Stressgen's StressXpress™ 8-iso-$PGF_{2\alpha}$ (direct) ELISA Kit provides a method for the quantitative determination of 8-iso-Prostaglandin $F_{2\alpha}$ in biological fluids. The kit is a competitive immunoassay. The assay uses a rabbit polyclonal antibody specific for 8-iso-$PGF_{2\alpha}$ to bind competitively to either 8-iso-$PGF_{2\alpha}$ in the sample or to 8-iso-$PGF_{2\alpha}$ covalently attached to alkaline phosphatase. After a simultaneous incubation at either room temperature or 4°C, the excess reagents are washed away and p-nitrophenly phosphate substrate is added. The enzyme reaction is stopped with an acid stop solution which converts the end point color to yellow. The intensity of the bound yellow color is inversely proportional to the concentration of 8-iso-$PGF_{2\alpha}$ in either standards or samples and is measured on a microplate reader at 405nm. 8-iso-$PGF_{2\alpha}$ concentrations of the samples are quantitated by interpolating absorbance readings from a standard curve generated with the calibrated 8-iso-$PGF_{2\alpha}$ standard provided.

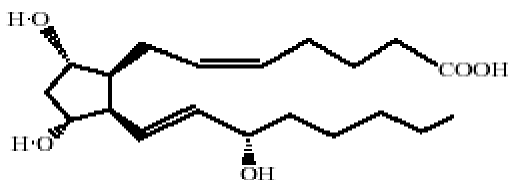
## Scientific Overview

8-epimer of Prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) is a major isoprostane that is mainly produced by non-enzymatic free radical-induced perioxidation of arachidonic acid (AA) present in phospholipids[1,2,3]. 8-iso-$PGF_{2\alpha}$ is also produced as a minor product of the COX-1 enzyme in human platelets[4] and the COX-2 isoform in human monocytes[5].

8-iso-$PGF_{2\alpha}$ has been found to be a potent constrictor of the renal and pulmonary vasculature and a broncoconstrictor[6,7]. It mediates smooth muscle cell growth, activates platelets[8] and induces derangement of the endothelial cell barrier function[9]. 8-iso-$PGF_{2\alpha}$ has also been suggested to participate as a pathophysiological mediator and to modify the fluidity and integrity of membranes[10].

8-iso-$F_{2\alpha}$ has been shown to circulate in plasma and be excreted in urine[11]. Elevated plasma, serum and urine levels of 8-iso-$PGF_{2\alpha}$ have been shown to be associated with cardiovascular risk factors such as cigarette smoking, hypercholesterolemia[12] and hyperhomocysteinemia[13]. Enhanced urinary excretion of 8-iso-$PGF_{2\alpha}$ has been described in association with both type 1 and type 2 diabetis mellitus and correlated with impaired glycemic control[14]. Elevated plasma content of free 8-iso-$F_{2\alpha}$ has been reported in women with preeclampsia and recent data indicates that 8-iso-$PGF_{2\alpha}$ may have a potential role in reduced trophoblast invasion in this condition[15, 16, 17].

### 8-iso-Prostaglandin $F_{2\alpha}$




## Precautions

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.

- Some components in this kit contain azide, which may react with lead or copper plumbing. Always flush with large volumes of water when disposing of reagents to prevent azide build-up.
- The Stop Solution (part# 210-P9) is a solution of trisodium phosphate. This solution is caustic, please handle with care and keep tightly capped.
- The activity of the alkaline phosphatase conjugate is dependent on the presence of $Mg^{2+}$ and $Zn^{2+}$ ions and is also affected by concentrations of chelators (>10mM), such as EDTA and EGTA.
- The 8-iso-$PGF_{2\alpha}$ Standard (part# 210-P7) provided is formatted in ethanolic buffer at a pH optimized to maintain 8-iso-$PGF_{2\alpha}$ integrity. Due to the possible known and unknown effects of prostaglandins, please handle this material with care.
- The kit's performance has been tested with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results.
- The components in each kit lot # have been quality assured and warranted in this specific combination only; please do not mix them with components from other kit lot #s.
- Please read the complete kit insert before performing this assay.

## Materials Provided

The StressXpress™ 8-iso-$PGF_{2\alpha}$ (direct) ELISA Kit contains the following components in sufficient quantities for 96 wells.

| Part Number | Component | Size | Description |
|---|---|---|---|
| 210-P1 | Goat anti-Rabbit IgG Microtiter Plate | 96 well plate | 12x8 removable strips and frame. Pre-coated plate with goat polyclonal antibody specific for rabbit IgG. |
| 210-P2 | 8-iso-$PGF_{2\alpha}$ Direct Conjugate | 5mL | Blue solution of 8-iso-$PGF_{2\alpha}$ conjugated to alkaline phosphatase. |
| 210-P3 | 8-iso-$PGF_{2\alpha}$ ELISA Antibody | 5mL | Yellow solution of Rabbit polyclonal antibody specific for 8-iso-$PGF_{2\alpha}$. |
| 210-P4 | Direct 8-iso-$PGF_{2\alpha}$ Sample Diluent | 30mL | Buffer to dilute standards and samples. Contains sodium azide as a preservative. |
| 210-P5 | Neutralizing Reagent | 6mL | |
| 210-P6 | Wash Buffer Concentrate | 30mL | Concentrated solution of Tris buffered saline containing detergents and sodium azide as a preservative. |
| 210-P7 | 8-iso-$PGF_{2\alpha}$ Standard | 0.5mL | 1,000,000 pg/mL solution of 8-iso-$PGF_{2\alpha}$ |
| 210-P8 | p-Npp Substrate | 20mL | Ready to use solution of p-nitrophenyl phosphate in buffer. |
| 210-P9 | Stop Solution | 5mL | Solution of trisodium phosphate in water. **Caution**: Caustic. Keep tightly capped. |
| 210-P10 | Plate Sealer | 1 | Adhesive sealer to cover plate. |

## Storage of Materials

All components of this kit are stable at 4°C until the kit's expiration date.

Unused wells of the Goat anti-Rabbit IgG Microtiter Plate should be resealed in the foil bag provided with desiccant and stored at 4°C.

## Materials Required but Not Provided

- Deionized or distilled water
- 10N Base (NaOH or KOH)
- Concentrated HCl
- Precision pipettes capable for accurately delivering volumes between 5 µL and 1,000 µL
- Repeater pipettes for delivering 50 µL and 200 µL
- A disposable beaker for diluting buffer concentrates.
- Graduated cylinders
- 45°C Water bath or incubator
- Microcentrifuge
- A microplate shaker
- Adsorbent paper for blotting
- Microplate reader capable of reading at 405 nm, preferably with correction between 570 and 590 nm

## Critical Assay Parameters and Notes

- This kit contains a pre-coated microtiter plate with removable wells to allow assaying on separate occasions. Unused wells must be kept desiccated at 4°C in the sealed foil bag. The wells should be used in the frame provided.
- Urine is not a suitable sample for analysis in this kit. Urine samples should be measured using the 8-iso $PGF_{2\alpha}$ ELISA Kit (Catalog # EKS-200).
- Exercise appropriate laboratory safety precautions when performing this assay.
- Allow reagents to warm to room temperature at least 30 minutes prior to opening.
- **Care must be taken to minimize contamination by endogenous alkaline phosphatase**. Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands.
- To avoid cross contamination, change disposable pipette tips between the addition of each standard, samples, and reagents and add the reagents to the side of the wells. Use separate reagent troughs/reservoirs for each reagent.
- This assay requires pipetting of small volumes. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated and pipette tips are pre-rinsed with the reagent.
- Pipet standards and samples to the bottom of the wells.
- **Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.**
- The components in each kit lot # have been quality assured and warranted in this specific combination only; please do not mix them with components from other kit lot #s. It is recommended that the reagents are not used beyond the kit expiration date.
- Run both standards and samples in duplicate.
- Include a standard curve each time the assay is performed.
- Standards can be made up in either glass or plastic tubes.

## Sample Preparation

Hydrolysis of lipoprotein or phospholipid coupled 8-iso-Prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) is required to ensure that the measured 8-iso-$PGF_{2\alpha}$ is a true reflection of both free and esterified isoprostane. To hydrolyze the ester bond the sample is treated with 2N base and then neutralized with acid. See below for details. **It is important that all standards and diluted samples are in the same matrix and that all dilutions of samples and standards are made with the Direct 8-iso-$PGF_{2\alpha}$ Sample Diluent (part# 210-P4) solution provided.**

**Note:** *Hydrolysis will destroy some of the liberated 8-iso-$PGF_{2\alpha}$ due to the effects of strong base on the isoprostane structure. However, the relative change in 8-iso-$PGF_{2\alpha}$ should be identical from sample to sample. If you wish to determine the percentage of endogenous 8-iso-$PGF_{2\alpha}$ destroyed during hydrolysis, add a known amount of 8-iso-$PGF_{2\alpha}$ to a sample prior to hydrolysis and determine the percent recovery of added 8-iso-$PGF_{2\alpha}$.*

**1. Tissue Samples**

- Prior to hydrolysis, samples should be stored at -20°C or lower and tissue samples should be powdered.
- Homogenization or other methods of cell disruption may be used.
- An appropriate excess volume of 2N base should be used. We recommend 1mL of 2N NaOH or KOH for every 10 µg-1mg of tissue.
- Cover samples and heat at 45°C for 2 hours to ensure hydrolysis.
- After hydrolysis, cool samples and treat with an equal volume of 2N HCl. For example, if 2 mg of tissue are hydrolyzed in 2 mL of 2N NaOH, 2 mL of 2N HCl would be added after the hydrolysis step to neutralize the base.
- Centrifuge the neutralized samples at 3,000 rpm in a microcentrifuge.

- If necessary, check the pH of the neutralized samples. The pH should be in the range of 6-8. The clear supernatant can be used in the assay or stored at ≤-20°C for future use.

**Note:** *Dilute samples with the Direct 8-iso-$PGF_{2\alpha}$ Sample Diluent prior to analysis.*

**2. Serum, EDTA or heparinized Plasma**

- Prior to hydrolysis, samples should be kept frozen at -20°C or lower.
- Use 1 part of 10N NaOH for every 4 parts of liquid sample.
- Cap and heat at 45°C for 2 hours.
- Cool, then add 100 µL of concentrated HCl per 500 µL of hydrolyzed sample. The sample should turn milky after this addition.
- Centrifuge the samples for 5 minutes at 14,000 rpm in a microcentrifuge.
- If necessary, check the pH of the neutralized samples. The pH should be in the range of 6-8. The clear supernatant can now be used in the assay or stored at ≤ -20°C for future use.

**Note:** *Consult the Sample Recovery section on page 10 for the appropriate dilution of the hydrolyzed, neutralized sample with the Direct 8-iso-$PGF_{2\alpha}$ Sample Diluent solution prior to analysis.*

*Remember to correct any measured 8-iso-$PGF_{2\alpha}$ concentrations for the dilution of the original sample by taking into account added base used for hydrolysis and the HCl used for neutralization. Based on this protocol, the total dilution for your liquid samples would be 1:1.5.*

## Reagent Preparation

***Diluted standards should be used within 60 minutes of preparation.***

**1. 8-iso-$PGF_{2\alpha}$ Standard (part# 210-P7)**

a) Allow the 8-iso-$PGF_{2\alpha}$ Standard solution to warm to room temperature.
b) Label five (5) 12 x 75 mm glass Tubes #1 through #5.
c) Add 900 µL of Direct 8-iso-$PGF_{2\alpha}$ Sample Diluent into Tube #1.
d) Add 800 µL of Direct 8-iso-$PGF_{2\alpha}$ Sample Diluent into Tubes #2 through #5.
e) Add 100 µL of the 8-iso-PGF2α Standard (1,000,000 pg/mL) to Tube #1 (100,000 pg/mL). Vortex thoroughly.
f) Add 200 µL of Tube#1 (100,00 pg/mL) to Tube #2 (20,000 pg/mL). Vortex thoroughly.
g) Similarly, complete the dilution series to generate the remaining standards (200 µL from Tube #2 to Tube #3, vortex thoroughly, etc) up to and including tube#5.

**Note:** The concentration of 8-iso-$PGF_{2\alpha}$ in Tubes #1 through #5 will be 100,000, 20,000, 4,000, 800 and 160 pg/mL respectively. The diluted standards should be used within 60 minutes of preparation.

**2. Wash Buffer Concentrate (part# 210-P6)**

a) Allow the Wash Buffer Concentrate to warm to room temperature.
b) Dilute the 30mL of Wash Buffer Concentrate with 270mL of deionized water. This can be stored at room temperature for up to 3 months.

## Assay Procedure

*Allow all reagents to warm to room temperature for at least 30 minutes prior to opening.*
*All standards and samples should be run in duplicate.*

1. Determine the required number of wells to be used and remove excess wells from the frame and reseal with the desiccant in the foil pouch provided. Store unused wells at 4°C.

2. Add 50 µL of Neutralizing Reagent into each well, except the Total Activity (TA) and Blank wells.

3. Add 50 µL of Direct 8-iso-$PGF_{2\alpha}$ Sample Diluent into the Non-specific binding (NSB) and the Bo (0 pg/mL Standard) wells.

4. Add 50 µL of Standards #1 through #5 into the appropriate wells.

5. Add 50 µL of the hydrolyzed/neutralized samples into the appropriate wells.

6. Add another 50 µL of Direct 8-iso-$PGF_{2\alpha}$ Sample Diluent into the NSB wells.

7. Add 50 µL of blue 8-iso $PGF_{2\alpha}$ Direct Conjugate into each well, except the Total Activity (TA) and Blank wells.

8. Add 50 µL of yellow 8-iso $PGF_{2\alpha}$ ELISA Antibody into each well, except the Blank, TA and NSB wells.

**Note:** *Every well used should be **Brown** in color except the NSB wells which should be **Purple**. The Blank and TA wells are empty at this point and have no color.*

9. Incubate the plate at room temperature on a plate shaker for 2 hours at ~500 rpm or 18-24 hours at 4°C. Cover the plate with the plate sealer provided, if so desired.

10. Empty the contents of the wells and wash by adding 200 µL of wash solution to each well. Repeat the wash 2 more times for a total of **3 Washes.**

11. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

12. Add 5 µL of the blue 8-iso-$PGF_{2\alpha}$ Direct Conjugate to the TA wells.

13. Add 200 µL of the p-Npp Substrate solution to each well. Incubate at room temperature for 45 minutes without shaking.

14. Add 50 µL of the Stop Solution to each well. This stops the reaction and the plate should be read immediately.

15. Blank the plate reader against the Blank wells, read the absorbance at 405nm., preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean absorbance of the blank wells from all readings.

## Calculation of Results

1. Calculate the average Net Optical Density (OD) bound for each standard and sample by subtracting the average NSB OD from the average OD bound:

   Average Net OD = Average Bound OD - Average NSB OD

2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

   $$\text{Percent Bound} = \frac{\text{Net OD}}{\text{Net Bo OD}} \times 100$$

3. To generate the standard curve, plot the 8-iso-$PGF_{2\alpha}$ Standard Concentration (pg/mL) on the X-axis using a log scale and the Percent Bound (%) on the Y-axis. Determine the best fit line.

4. Interpolate the sample concentrations of 8-iso-$PGF_{2\alpha}$ from the standard curve. Correct any measured 8-iso-$PGF_{2\alpha}$ concentrations for the dilution of the original sample by addition of the base used for hydrolysis, and the HCl used for neutralization.

**Note:** *Manufacturers of microplate readers usually offer accompanying software programs that will analyze data, plot standard curves and calculate sample concentrations. To set up the program for calculating the results, consult with the software instruction manual or contact the manufacturer of the microplate reader.*

## Typical Results

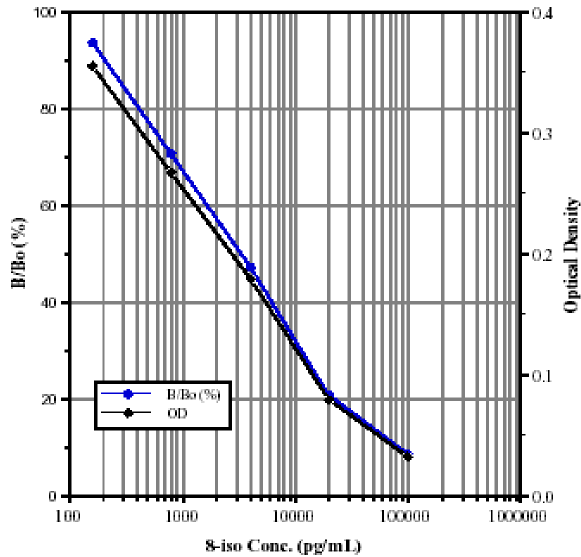
The results shown below are for illustration only and **should not** be used to calculate results.

| | **2 Hour Format** | | | **Overnight Format** | | |
|---|---|---|---|---|---|---|
| Sample | Average Net OD | Percent Bound | **8-iso-$PGF_{2\alpha}$ (pg/mL)** | Average Net OD | Percent Bound | **8-iso-$PGF_{2\alpha}$ (pg/mL)** |
| Blank OD | (0.074) | | | (0.092) | | |
| TA | 0.338 | | | 0.275 | | |
| NSB | 0.000 | 0.00% | | 0.000 | 0.00% | |
| Bo | 0.451 | 100% | **0** | 0.405 | 100% | |
| S1 | 0.033 | 7.3% | **100,000** | 0.025 | 6.2% | **100,000** |
| S2 | 0.079 | 17.5% | **20,000** | 0.071 | 17.5% | **20,000** |
| S3 | 0.179 | 39.7% | **4,000** | 0.158 | 39.0% | **4,000** |
| S4 | 0.268 | 59.4% | **800** | 0.251 | 61.9% | **800** |
| S5 | 0.356 | 78.9% | **160** | 0.329 | 81.2% | **160** |
| Unknown 1 | 0.282 | 62.5% | **683** | 0.084 | 20.7% | **15,604** |
| Unknown 2 | 0.117 | 25.9% | **10,454** | 0.232 | 57.3% | **1,145** |

## Typical Standard Curves

The standard curves shown below **must not** be used to calculate 8-iso-$PGF_{2\alpha}$ concentrations; each user must run a standard curve for each plate used.

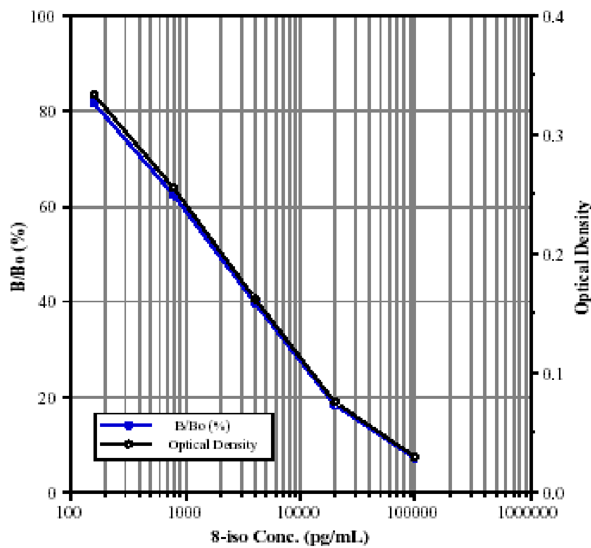
**2 Hour Format**




### Typical Quality Control Parameters

| | | |
|---|---|---|
| Total Activity Added | = | 0.338 x 10 = 3.38 |
| %NSB | = | -0.031% |
| %Bo/TA | = | 1.3% |
| Quality of Fit | = | 0.998 (Calculated from 4 parameter logistic curve fit) |
| 20% Intercept | = | 24,810.5 pg/mL |
| 50% Intercept | = | 3,019.6 pg/mL |

**Overnight Format**




### Typical Quality Control Parameters

| | | |
|---|---|---|
| Total Activity Added | = | 0.257 x 10 = 2.57 |
| %NSB | = | -0.304% |
| %Bo/TA | = | 1.33% |
| Quality of Fit | = | 0.999 (Calculated from 4 parameter logistic curve fit) |
| 20% Intercept | = | 16,031 pg/mL |
| 50% Intercept | = | 1,532 pg/mL |

## Performance Characteristics

**Sensitivity**
To determine the sensitivity of this assay, the mean optical density value for 16 replicates of Bo (0 pg/mL Standard) were compared to the mean optical density for 16 replicates of Standard #5 (160 pg/mL). The detection limit was determined as the concentration of 8-iso-$PGF_{2\alpha}$ measured at two standard deviations from the zero along the standard curve.
The sensitivity of this assay was determined to be 103.2 pg/mL for the 2 hour format and 40.0 pg/mL for the overnight format.

**Linearity**
To determine linearity, a sample containing 50,000 pg/mL 8-iso- $PGF_{2\alpha}$ was serially diluted 7 times 1:2 in Direct 8-iso-$PGF_{2\alpha}$ Sample Diluent and measured in this assay. The data was plotted graphically as actual 8-iso-$PGF_{2\alpha}$ concentration versus measured 8-iso-$PGF_{2\alpha}$ concentration.
The line obtained had a slope of 0.967 and a correlation coefficient of 0.986.

**Precision**
Intra-Assay Precision (Within Run Precision)
To determine intra-assay precision, three samples of low, medium and high 8-iso-$PGF_{2\alpha}$ concentrations were assayed sixteen times on one plate. The intra-assay coefficient of variation was determined to be <12%.

Inter-assay Precision (Between Run Precision)
To determine inter-assay precision three samples of low, medium and high 8-iso-$PGF_{2\alpha}$ concentrations were assayed in four individual assays. The inter-assay coefficient of variation was determined to be <11%.

| | 8-iso-$PGF_{2\alpha}$ (pg/mL) | Intra-assay %CV | Inter-assay %CV |
|---|---|---|---|
| Low | 5,092 | 5.1 | |
| Medium | 3,264 | 5.7 | |
| High | 970 | 11.3 | |
| Low | 1,473 | | 5.4 |
| Medium | 4,501 | | 5.8 |
| High | 6,679 | | 10.4 |

**Cross Reactivities**
To determine the cross reactivities for a number of related eicosanoid compounds in this assay, the cross reactant (purity checked by NMR and other analytical methods) was dissolved in assay buffer at concentrations from 100,000 to 6 pg/mL and measured in the 8-iso-$PGF_{2\alpha}$ (direct) assay. The measured 8-iso-$PGF_{2\alpha}$ concentration at 50% B/Bo was calculated and compared to the actual concentration of cross reactant in the sample and expressed as a percentage.

| Compound | Cross Reactivity |
|---|---|
| 8-iso-$PGF_{2\alpha}$ | 100% |
| $PGF_{1\alpha}$ | 11.85% |
| $PGF_{2\alpha}$ | 0.44% |
| $PGE_1$ | 0.04% |
| $TXB_2$ | 0.009% |
| 13,14-dihydro-15-keto-$PGF_{2\alpha}$ | 0.008% |
| $PGB_1$ | 0.008% |
| $PGE_3$ | 0.005% |
| 8-iso-$PGE_1$ | <0.001% |
| $PGA_2$ | <0.001% |
| 6,15-keto-13,14-dihydro-$PGF_{1\alpha}$ | <0.001% |
| 6-keto-$PGF_{1\alpha}$ | <0.001% |
| $PGJ_2$ | <0.001% |
| 2-Arachidonoylglycerol | <0.001% |
| Anandamide | <0.001% |

## Sample Recoveries

To determine sample recovery, 8-iso-$PGF_{2\alpha}$ was spiked into the three undiluted hydrolysed samples listed below which were neutralized and diluted into the Direct 8-iso Sample Diluent and then assayed in the kit. The % recovery was calculated to be >90% for all three samples.

| Sample | % Recovery* | Recommended Dilution* |
|---|---|---|
| Porcine Serum | 93.8 | None |
| Equine Heparinized Plasma | 97.2 | None |
| Porcine EDTA Plasma | 109.8 | 1:8 |

* See Sample Preparation instructions on page 4 for details.

**Note:** *Urine is not a suitable sample for analysis in this kit. Urine samples should be measured using the 8-iso-$PGF_{2\alpha}$ ELISA Kit, (Catalog # EKS-200).*

## References


1. Zeldin, D.C. (2001) *J. Biol. Chem*. **276:** 36059-36062.
2. Reilly, M.P., Lawson, J.A. and FitzGerald, G.A. (1998) *J. Nutr*. **128**: 434S-438S.
3. Funk, C.D. (2001) *Science* **294**: 1871-1875.
4. Pratico, D., Lawson, J.A. and FitzGerald, G.A. (1995) *J. Biol. Chem*. **270**: 9800-9808.
5. Pratico, D. and FitzGerald, G.A. (1996) *J. Biol. Chem*. **271**: 8919-8924.
6. Takahashi, K., Nammour, T.M., Fukunaga, M., Ebert, J., Morrow, J.D., Roberts, L.J.II., Hoover, R.L. and Badr, K.F. (1992) *J. Clin. Invest*. **90**: 136-141.
7. Kang, K.H., Morrow, J.D., Roberts, L.J.II., Newman, H. and Banerjee, M. (1993) *J. Appl. Physiol*. **74**: 460-465.
8. Patrono, C. and Fitzgerald, G. A. (1997) *Arterioscler. Thromb. and Vasc. Biol.* **17:** 2309-2315.
9. Hart, C. M., Karman, R. J., Blackburn, T. L., Gupta, M. P., Garcia, J. G. and Mohler, E. R. (1998) *Prostaglandins Leukot. Essent. Fatty Acids* **58:** 9-16.
10. Yura, T., Fukunaga, M., Grygorczyk, R., Makita, N., Takahashi, K and Badr, K. F. (1995) *Adv. Prostaglandin Thromboxane Leukot. Res*. **23**: 237-239.
11. Helmersson, J. and Basu, S. (2001) *Prostaglandins Leukot. Essent. Fatty Acids* **65**: 99-102.
12. Reilly, M. P., Protico, D., Delanty, N., DiMinno, G., Tremoli, E., Rader, D., Kapoor, S., Rokach, J., Lawson, J. and FitzGerald, G. A. (1998) *Circulation* **98:** 2822-2828.
13. Voutilainen, S., Morrow, J. D., Roberts, J. II., Alfthan, G., Alho, H., Nyyssonen, K. and Salonen, J. T. (1999) *Arterioscler. Thromb. Vasc. Biol*. **19**: 1263-1266.
14. Mezzetti, A., Cipollone, F. and Cuccurullo, F. (2000) *Cardiovasc. Res*. **47**:475-488.
15. Barden, A., Beilin, L.J., Ritchie, J., Croft, K.D., Walters, B.N. and Michael, C.A. (1996) *Clin. Sci*. **91**: 711-718.
16. Staff, A.C., Ranheim, T., Henriksen, T. and Halvorsen, B. (2000) *Hypertension* **35**: 1307-1313.
17. Halvorsen, B., Staff, A.C., Henriksen, T., Sawamura, T. and Ranheim, T (2001) *Hypertension* **37**: 1184-1190.


05/03/02 v.1