# Table of Contents

2 Introduction  
2 Principle  
3 Materials Supplied  
3 Storage  
3 Materials Needed but Not Supplied  
4 Reagent Preparation  
5 Sample Handling  
5 Sample Recoveries  
6 Assay Procedure  
6 Calculation of Results  
7 Typical Results  
8 Performance Characteristics  
10 Interferences  
11 References  
12 Limited Warranty

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

**> Cortisol FPIA kit**  
Catalog # ADI-920-071  
Fluorescence Polarization Immunoassay Kit  
For use with cell supernatants

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All reagents should be stored at 4°C  
Check our website for additional protocols, technical notes and FAQs  
For proper performance, use the insert provided with each individual kit received
Introduction

Cortisol (hydrocortisone, compound F) is a steroid hormone synthesized from cholesterol and secreted by the adrenal cortex. Found in the blood either as free cortisol or bound to corticosteroid-binding globulin (CBG), serum levels are highest in the morning and decrease throughout the day\(^1^,2\). Metabolically, cortisol promotes gluconeogenesis, liver glycogen deposition, and the reduction of glucose utilization\(^1\). Cortisol also functions as an important anti-inflammatory and plays a role in hypersensitivity, immunosuppression and disease resistance. It has been shown that plasma cortisol levels elevate in response to stress\(^1^,3\). Abnormal cortisol levels are being linked to a variety of physiological conditions including prostate cancer\(^4\), depression\(^5\), schizophrenia\(^6\) and Cushing’s Syndrome\(^7\).

Principle

1. Samples and standards are added to uncoated wells.

2. A solution of cortisol covalently conjugated to fluorescein is then added to the wells.

3. A solution of polyclonal antibody to cortisol is next added. This binds, in a competitive manner, the cortisol in the standard, sample or conjugate.

4. The plate is incubated at room temperature for at least 30 minutes. The FP signal is stable for at least 20 hours.

5. The plate is then read at 520 - 535 nm, with excitation at 485 nm. The amount of signal is inversely proportional to the concentration of cortisol in the standards or samples.
**Materials Supplied**

1. **Cortisol FPIA Antibody**  
   8 mL, Catalog No. 80-1454  
   A solution of polyclonal antibody to cortisol.

2. **Assay Buffer Concentrate**  
   30 mL, Catalog No. 80-2088  
   Tris buffered saline containing proteins and sodium azide as preservative.

3. **Cortisol FPIA Conjugate Concentrate**  
   0.1 mL, Catalog No. 80-1453  
   A solution of fluorescein conjugated to cortisol.

4. **Cortisol Standard**  
   0.5 mL, Catalog No. 80-1452  
   A solution of 8,000,000 pg/mL cortisol.

**Storage**

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

**Materials Needed but Not Supplied**

1. Solid black uncoated low-binding microtiter plate.  
2. Foil microtiter plate sealer, if desired.  
3. Fluorescence polarization detector, such as a Dynex Triad Multimode, capable of reading emissions at 520 - 535 nm, with excitation at 485 nm.
Reagent Preparation

1. **Assay Buffer**
   Prepare the assay buffer by diluting 25 mL of the supplied Assay Buffer 1 Concentrate with 225 mL of deionized water. This can be stored at room temperature until the kit’s expiration, or for 3 months, whichever is earlier. Use the diluted assay buffer for the entire assay.

2. **Cortisol Conjugate**
   Count the total number of wells that will receive conjugate. Use the following formula to calculate the volume of Cortisol Conjugate Concentrate and the assay buffer needed to prepare cortisol conjugate.

   A. \((\text{Number of wells} + 1) \times 0.05 \text{ mL} / \text{well} = \text{Volume of assay buffer needed}\). Increase the calculated volume to the next whole milliliter.

   B. \((\text{Volume from part A}) \times 10 \mu\text{L} / \text{mL} = \text{Volume of Cortisol Conjugate Concentrate needed}\).

   Pipet the volume of assay buffer from part A into an amber container. From this volume, remove the volume calculated in part B. Add the calculated Cortisol Conjugate Concentrate to the assay buffer. Vortex thoroughly and use.

4. **Cortisol Standards**
   Diluted standards should be used within 60 minutes of preparation.
   Allow the 8,000,000 pg/mL standard stock to warm to room temperature. Label six 12 x 75 tubes #1 through #6. Pipet 900 µL of the assay buffer into tube #1. Pipet 750 µL of the assay buffer into tubes #2 through #6. Add 100 µL of the 8,000,000 pg/mL Cortisol Standard into tube #1 and vortex thoroughly. Add 250 µL of tube #1 to tube #2 and vortex thoroughly. Add 250 µL of tube #2 to tube #3 and vortex thoroughly. Continue this for tubes #4 through #6. The concentrations of cortisol in the tubes are labeled above.
**Sample Handling**

The Cortisol FPIA kit is compatible with cortisol samples in defined buffers and cell culture media. Samples diluted sufficiently into the assay buffer can be read directly from the standard curve. Samples containing some organic solvents or inherently fluorescing materials may interfere with the assay. Please refer to the Interferences section on page 10 for details.

**Sample Recoveries**

Cortisol standard was spiked into the following buffers, which were already diluted with the assay buffer, and measured in the kit. The results are shown below. These are the minimum dilutions required to remove the matrix interference of these solutions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Recovery</th>
<th>Recommended Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hanks Buffered Saline Solution</td>
<td>104%</td>
<td>≥ 1:8</td>
</tr>
<tr>
<td>RPMI-1640 without phenol red</td>
<td>102%</td>
<td>≥ 1:8</td>
</tr>
<tr>
<td>RPMI-1640 with phenol red, sodium pyruvate and</td>
<td>112%</td>
<td>≥ 1:32</td>
</tr>
<tr>
<td>Non-Essential Amino Acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPMI-1640 with phenol red, 10% FBS and antibiotics</td>
<td>107%</td>
<td>≥ 1:32</td>
</tr>
</tbody>
</table>

Samples must be stored at or below -20°C to avoid loss of bioactive analyte. Repeated freeze/thaw cycles should be avoided.
Assay Procedure

The following procedure was used to generate all performance data cited in this publication.

1. Pipet 150 µL of assay buffer into the Total Fluorescence (TF) wells.
2. Pipet 100 µL of assay buffer into the Bo (0 pg/mL) wells.
3. Pipet 100 µL of Standards #1 through #6 to the bottom of the appropriate wells.
4. Pipet 100 µL of the samples to the bottom of the appropriate wells.
5. Add 50 µL of the conjugate into each well.
6. Add 50 µL of antibody into each well, except the TF wells.
7. Seal the plate with a foil plate sealer. Incubate the plate for at least 30 minutes at room temperature. The FP signal is stable for at least 20 hours.
8. Read plate on a suitable fluorescence polarization detector at 520 - 535 nm emission, with excitation at 485 nm, using the appropriate settings for that instrument.

Calculation of Results

Several options are available for the calculation of the concentration of cortisol in samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentrations can be calculated as follows:

1. Calculate the binding for each standard and sample as a percentage of the maximum binding (Bo), using the following formula:

   \[
   \text{Percent Bound} = \frac{\text{Average } mP}{\text{Average } Bo} \times 100
   \]

2. Using Logit-log paper, plot the Percent Bound for each standard versus cortisol concentration in each standard. Approximate a straight line through the points. The concentration of the unknowns can be determined by interpolation.

   Samples with concentrations outside of the standard curve range will need to be re-analyzed using a higher dilution.
**Typical Results**

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Net mP</th>
<th>Percent Bound</th>
<th>Cortisol (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>64</td>
<td>31.0%</td>
<td>800,000</td>
</tr>
<tr>
<td>S2</td>
<td>82</td>
<td>39.9%</td>
<td>200,000</td>
</tr>
<tr>
<td>S3</td>
<td>108</td>
<td>52.9%</td>
<td>50,000</td>
</tr>
<tr>
<td>S4</td>
<td>140</td>
<td>68.5%</td>
<td>12,500</td>
</tr>
<tr>
<td>S5</td>
<td>177</td>
<td>86.2%</td>
<td>3,125</td>
</tr>
<tr>
<td>S6</td>
<td>190</td>
<td>92.8%</td>
<td>781</td>
</tr>
<tr>
<td>Unknown 1</td>
<td>83</td>
<td>40.4%</td>
<td>171,246</td>
</tr>
<tr>
<td>Unknown 2</td>
<td>166</td>
<td>81.0%</td>
<td>4,624</td>
</tr>
<tr>
<td>Bo</td>
<td>205</td>
<td>100%</td>
<td>0</td>
</tr>
</tbody>
</table>

![Graph showing cortisol concentration vs. %B/Bo and mP](image-url)
Performance Characteristics

Specificity

The cross reactivities for a number of related compounds were determined by diluting the compounds in the kit assay buffer at concentrations from 8,000,000 to 800 pg/mL. These samples were then measured in the Cortisol FPIA, and the measured cortisol concentration at 50% B/Bo calculated. The % cross reactivity was calculated by comparison with the actual concentration of the cross reactant in the sample and expressed as a percentage.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-Deoxycortisol</td>
<td>14%</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>13%</td>
</tr>
<tr>
<td>Cortisone</td>
<td>9.1%</td>
</tr>
<tr>
<td>17α-Hydroxyprogesterone</td>
<td>2.5%</td>
</tr>
<tr>
<td>17-Hydroxypregnenolone</td>
<td>2.5%</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>2.0%</td>
</tr>
<tr>
<td>Progesterone</td>
<td>1.1%</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>0.5%</td>
</tr>
<tr>
<td>Prednisone</td>
<td>0.5%</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>&lt;0.1%</td>
</tr>
<tr>
<td>Testosterone</td>
<td>&lt;0.1%</td>
</tr>
</tbody>
</table>

Z-Factor

The Z-Factor is a dimensionless statistic that reflects the dynamic signal range and variation of an assay. This provides a useful parameter to evaluate the robust quality of a given assay. The following formula was used:

\[
Z\text{-Factor} = 1 - \left( \frac{(3 \times 1SD \text{ of Positive}) + (3 \times 1SD \text{ of Negative})}{\text{Positive} - \text{Negative}} \right)
\]

The Z-Factor of the assay was determined to be 0.823.
**Sensitivity**

The sensitivity of the assay, defined as the concentration of cortisol measured at 2 standard deviations from the mean of 24 zeros along the standard curve, was determined to be 685 pg/mL.

**Linearity**

A buffer sample containing cortisol was serially diluted 1:2 in the kit assay buffer and measured in the assay. The results are shown in the table below.

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Expected (pg/mL)</th>
<th>Observed (pg/mL)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat</td>
<td>---</td>
<td>445,648</td>
<td>---</td>
</tr>
<tr>
<td>1:2</td>
<td>222,824</td>
<td>282,570</td>
<td>127%</td>
</tr>
<tr>
<td>1:4</td>
<td>111,412</td>
<td>112,481</td>
<td>101%</td>
</tr>
<tr>
<td>1:8</td>
<td>55,706</td>
<td>56,639</td>
<td>102%</td>
</tr>
<tr>
<td>1:16</td>
<td>27,853</td>
<td>28,787</td>
<td>103%</td>
</tr>
<tr>
<td>1:32</td>
<td>13,926</td>
<td>12,143</td>
<td>87%</td>
</tr>
<tr>
<td>1:64</td>
<td>6,963</td>
<td>6,842</td>
<td>98%</td>
</tr>
<tr>
<td>1:128</td>
<td>3,482</td>
<td>3,077</td>
<td>88%</td>
</tr>
<tr>
<td>1:256</td>
<td>1,741</td>
<td>1,752</td>
<td>101%</td>
</tr>
<tr>
<td>1:512</td>
<td>870</td>
<td>870</td>
<td>100%</td>
</tr>
</tbody>
</table>
Precision

Intra-assay precision was determined by assaying 24 replicates of three buffer controls containing cortisol in a single assay.

<table>
<thead>
<tr>
<th>pg/mL</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>189,777</td>
<td>27</td>
</tr>
<tr>
<td>24,592</td>
<td>21</td>
</tr>
<tr>
<td>4,947</td>
<td>20</td>
</tr>
</tbody>
</table>

Inter-assay precision was determined by measuring buffer controls of varying cortisol concentrations in multiple assays over several days.

<table>
<thead>
<tr>
<th>pg/mL</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>157,258</td>
<td>26</td>
</tr>
<tr>
<td>28,304</td>
<td>22</td>
</tr>
<tr>
<td>4,434</td>
<td>18</td>
</tr>
</tbody>
</table>

Interferences

Cortisol standard was spiked into the following solutions, which were already diluted with the assay buffer, and measured in the kit. The results were as follows:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Recovery</th>
<th>Recommended Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>112%</td>
<td>≤ 5%</td>
</tr>
<tr>
<td>DMSO</td>
<td>110%</td>
<td>≤ 2.5%</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>91%</td>
<td>≤ 1.3%</td>
</tr>
<tr>
<td>Ethanol</td>
<td>116%</td>
<td>≤ 1.3%</td>
</tr>
<tr>
<td>2-propanol</td>
<td>98%</td>
<td>≤ 0.6%</td>
</tr>
<tr>
<td>DMF</td>
<td>112%</td>
<td>≤ 0.2%</td>
</tr>
</tbody>
</table>
References

Use of Product

This product contains research chemicals. As such, they should be used and handled only by or under the supervision of technically qualified individuals. This product is not intended for diagnostic or human use.

Warranty

Enzo Life Sciences International, Inc. makes no warranty of any kind, expressed or implied, which extends beyond the description of the product in this brochure, except that the material will meet our specifications at the time of delivery. Enzo Life Sciences International, Inc. makes no guarantee of results and assumes no liability for injuries, damages or penalties resulting from product use, since the conditions of handling and use are beyond our control.

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