Prostaglandin E$_2$

$[^{125}\text{I}]$ FlashPlate® Assay

Catalog Number

SMP-003
(192 wells)

FOR LABORATORY USE

CAUTION: A research chemical for research purposes only.
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I. PROPRIETARY NAME

Prostaglandin E2 [125I] FlashPlate® Assay
NEN™ Life Science Products Catalog Number SMP-003

II. INTENDED USE

This product is designed to estimate levels of Prostaglandin E2 in biological fluids.

III. BACKGROUND INFORMATION

The schematic for the "in vivo" metabolic pathways of the major prostaglandins, shown in Figure 1, represents only the most general outline of a very complex series of biochemical interconversions. Linoleic acid is an essential fatty acid which is metabolized to arachidonic acid, the starting compound of the cascade. Arachidonic acid is stored in cell walls esterified in phospholipids (1). Upon demand, arachidonic acid may be released from the cell wall by phospholipase A2 (2).

Prostaglandin synthetase contains both cyclo-oxygenase and peroxidase activities to convert arachidonic acid to prostaglandin endoperoxide. Cyclo-oxygenase enzymatically metabolizes arachidonic acid to prostaglandin G2 (PGG2), a cyclic endoperoxide, while peroxidase reduces PGG2 to another cyclic endoperoxide, prostaglandin H2 (PGH2) (3, 4). Both PGG2 and PGH2 have a half-life of about five minutes at 37°C in aqueous buffer at pH 7.4 (5). Prostaglandin endoperoxide PGH2 is considered a pivotal compound, since it is metabolized by three different reaction pathways:

A. In the presence of thromboxane synthetase, known to be present in large amounts in platelets (4, 6), PGH2 is converted to thromboxane A2. Thromboxane A2 is rapidly hydrolyzed to thromboxane B2 (TXB2).

B. Prostacyclin synthetase, which has been demonstrated in the microsomal fraction of endothelial cells (7, 8), hydrolyzes PGH2 to prostacyclin. Prostacyclin is unstable and converts to 6-keto-prostaglandin F1α (6-keto-PGF1α).

C. Classical prostaglandins, PGE2, PGF2α, can be formed from the prostaglandin endoperoxide PGH2 either spontaneously or enzymatically in the absence of either thromboxane or prostacyclin synthetases (5). In addition, the classical prostaglandins may be formed in association with the other two pathways.
Prostaglandin E₂ (PGE₂) has been detected in plasma and urine as well as in a variety of human tissues (9).

IV PRINCIPLE OF THE PROCEDURE

The basic principle of this FlashPlate® Assay is competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. When unlabeled antigen from standards or samples and a fixed amount of tracer (labeled antigen) are allowed to react with a constant and limiting amount of antibody, decreasing amounts of tracer are bound to the antibody as the amount of unlabeled antigen is increased. See Figure 2.(10)
or unknown samples)

a_

Unlabeled Antigen-Antibody Complex
AgAb
A FlashPlate is a white 96 well polystrene microplate with plastic scintillator coated wells. In the Prostaglandin E2 \([^{125}I]\) FlashPlate Assay, the primary antibody is precoated on the walls of the microwells of a FlashPlate. The radioactive decay associated with the bound radiolabeled PGE2 causes a microplate surface scintillation effect detectable on a microplate scintillation counter. Separation of the bound from the free antigen is not necessary to quantitate the bound tracer.

After incubation the antigen-antibody complex is counted and the data is used to construct a standard (dose-response) curve from which the unknowns are obtained by interpolation. Aspiration of the contents of the wells is recommended if the plate is going to be kept at room temperature for greater than 10 minutes. Aspiration prevents re-equilibration of the plates to the "new" ambient temperature. Aspiration eliminates the need for color quench correction.

V. REAGENT DESCRIPTION

This kit is intended FOR LABORATORY USE. All necessary reagents are supplied for 192 (SMP-003) assay wells if the suggested assay protocol is followed.

<table>
<thead>
<tr>
<th>SMP-003</th>
<th>Kit Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 plates</td>
<td>PGE2 FlashPlate</td>
</tr>
<tr>
<td>2 vials</td>
<td>PGE2 ([^{125}I]) Tracer Concentrate, 0.75 mL</td>
</tr>
<tr>
<td>1 vial</td>
<td>PGE2 Standard Concentrate, 1.0 mL</td>
</tr>
<tr>
<td>1 bottle</td>
<td>Assay Buffer, 125 mL</td>
</tr>
</tbody>
</table>

The Prostaglandin E2 FlashPlate Assay is shipped on ice, but upon receipt the individual components should be stored as directed. Component stability and handling precautions are described below. Sodium azide has been added as an antibacterial agent where appropriate.

NOTE: The National Institute for Occupational Safety and Health has issued a bulletin citing the potentially explosive hazard due to the reaction of sodium azide with copper, lead, brass, or solder in plumbing systems. Although sodium azide is added at a minimal concentration, it is still recommended that copious amounts of water be used to flush the drain pipeline after disposal of these reagents in the plumbing system. Copper-free and lead-free discharge lines should be used whenever possible. Decontamination procedures should be followed prior to maintenance on drain lines which have been used for azide-containing reagents. Recommended decontamination procedures are available from RIA Technical Services.
A. PGE₂ [¹²⁵I] Tracer

The tracer concentrate contains < 2 µCi of PGE₂, [¹²⁵I]-, in 0.75 mL of acetonitrile. Stored at -20°C, the tracer concentrate is stable for at least one month from date of receipt. Immediately before use, dilute the tracer in Assay Buffer. The procedure for preparation of the working tracer solution appears in Section VI.C. Extreme care must be taken to avoid evaporation of the solvent which will result in changes in the concentration of the tracer. Avoid unnecessary exposure to air and ambient temperature. Use promptly, cap, and return to -20°C storage.

INSTRUCTIONS RELATING TO THE HANDLING, USE, STORAGE, AND DISPOSAL OF THIS RADIOACTIVE MATERIAL

This radioactive material may be received, acquired, possessed, and used only by research laboratories for in vitro laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use, and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

1. All radioactive materials must be labeled and secured in specifically designated posted areas. Records of receipt and survey must be maintained.

2. All work with these materials must be carried out only in authorized areas.

3. Prohibit mouth pipetting of radioactive materials.

4. There must be no smoking or eating within the work area.

5. Hands must be washed after handling radioactive materials.

6. Any spilled material must be wiped up quickly and thoroughly and the contaminated substances transferred to a suitable receptacle. The surfaces involved must be washed thoroughly with an appropriate decontaminant. Monitor to ensure the area has been effectively decontaminated.

7. When use of the Tracer reagent has been completed, empty and decontaminate the vial. This radioactive material can be discarded into the sanitary sewerage system using copious amounts of water to ensure a minimal discharge concentration.

8. Prior to disposal of the empty, uncontaminated Kit and Tracer containers to unrestricted areas, remove or deface the radioactive material labels or otherwise clearly indicate that the containers no longer contain radioactive material.
B. **PGE$_2$ Standard Concentrate**

This solution contains 100 ng/mL of prostaglandin E$_2$ in acetonitrile. Immediately before use in the assay, dilute an aliquot of the stock solution to prepare standards. A suggested procedure for preparing diluted standards appears in Section VI.B. Store the remaining standard concentrate at -20°C. Under these conditions, the solution is stable for at least two months. Extreme care must be taken to avoid evaporation of the solvent and resulting concentration of the standard. Avoid unnecessary exposure to air and ambient temperature. Use promptly, cap, and return to -20°C storage.

<table>
<thead>
<tr>
<th>WARNING</th>
</tr>
</thead>
<tbody>
<tr>
<td>STANDARDS</td>
</tr>
<tr>
<td>Toxic</td>
</tr>
<tr>
<td>Highly Flammable</td>
</tr>
<tr>
<td>EU</td>
</tr>
</tbody>
</table>

C. **Assay Buffer**

This solution contains stabilizers, detergent, protein and 0.05% sodium azide in sodium phosphate buffer, pH 6.8. Stored at 2 - 8°C, the assay buffer is stable for at least two months.

D. **PGE$_2$ FlashPlates**

Two microplates coated with solid scintillant to which anti-PGE$_2$ has been bound are supplied. Each plate is packaged in a sealed pouch and should not be opened until ready to use. The plate may be stored for at least two (2) months at 2-8°C.

E. **Topseal - A Plate Cover**

Four plate covers are provided with each kit.
VI. SAMPLE HANDLING

A. Collection and Storage

It is recommended that all samples be processed immediately after collection and assayed as soon as possible. PGE2 is reported to be stable in urine (11) if kept frozen, but significant decreases in PGE2 have been observed after plasma was stored at -20°C for a week.

Blood samples, at least 2 mL, should be collected in pre-chilled siliconized glass or polypropylene test tubes coated with a solution of 4.5mM EDTA containing a prostaglandin synthetase inhibitor such as indomethacin or aspirin (12). Indomethacin has been reported to be very effective at concentrations of up to 10 g/mL. The plasma fraction should be isolated from the whole blood as soon as possible after collection and frozen at -70°C if it is not assayed on the same day.

Urine should be stored at -20°C immediately after collection. Urinary PGE2 levels are reported to show a circadian rhythm, and a single sample may not be representative of the true value (13).

If tissues are not analyzed immediately after collection, they should be stored at -70°C or lower. Tissue samples should be processed in the presence of prostaglandin synthetase inhibitors (14), such as indomethacin, at concentrations up to 10 ug/mL.

It is good practice to assay, in each run, a method blank consisting of distilled water which has been extracted along with samples. This practice will assure the user that non-specific interfering substances have not been introduced from solvents, etc. It is also good practice to use [3H]-PGE2 (NET-428) as recovery marker in any extraction procedure.

1. Urine

PGE2 of renal origin is present in urine (15), in addition to at least eight metabolites derived from PGE2 in the peripheral circulation (16). Successful direct assay of urine for immunoreactive PGE2 levels has been reported (17).

The urine may be extracted by a modification of the method of Frolich (15) with good success. The urine sample is acidified to pH 4.0, and extracted two times with an equal volume of chloroform. The extracts are pooled, dried under nitrogen and reconstituted with assay buffer. It is recommended that serial dilutions be assayed because of the wide range of expected values. We have observed a reduction in blanks by filtering the reconstituted sample through a 0.22 um Millex™ filter.
2. **Tissue and Plasma**

Plasma and tissue extraction procedures have been reported in references 14, 18, and 19.

Due to the wide variety of sample types and extraction procedures used by our customers, validation of these procedures and any matrix effects remain the responsibility of the individual researcher.

**VII. PROCEDURE**

**A. Materials Required**

In addition to the reagents supplied with the kit, the following materials are required:

1. Pipettors and/or pipets that accurately and precisely deliver the required volumes. Pipets and/or pipet tips used to transfer diluted standards, tracer, or samples should be made of polypropylene or siliconized glass. Do not use those made of unsiliconized glass.

2. 96-Well Microplate Scintillation Counter

3. Polypropylene test tubes - 12x75 mm.

4. Test tube rack.

5. Laboratory vortex mixer.

6. Microplate shaker.

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Brand</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPC</td>
<td>Micromix</td>
<td>Form 20 Amp 4</td>
</tr>
<tr>
<td>Kanke &amp; Kunkel</td>
<td>IKA-Schuttler</td>
<td>350/min.</td>
</tr>
<tr>
<td>Lab-line Instruments</td>
<td>Titer Plate Shaker</td>
<td>4.5</td>
</tr>
<tr>
<td>New Brunswick</td>
<td>Gyrotory Shaker</td>
<td>350 RPM</td>
</tr>
</tbody>
</table>

* (The use of microplate shakers other than those listed above will need to be validated by the individual researcher.)

7. Radioactive waste container.

8. Aspiration apparatus: vacuum flasks, tubing, 8 channel manifold for microplates and vacuum pump.

9. 2-8°C refrigerator or equivalent.
B. Preparation of Prostaglandin E\textsubscript{2} Working Standards

An aliquot of the PGE\textsubscript{2} standard concentrate is diluted with assay buffer in order to prepare a series of working prostaglandin E\textsubscript{2} standards. A suggested dilution scheme to cover a standard curve range of 1.0 pg to 100 pg added (per 0.1 mL/well) plus a solution for creation of a non specific binding well is shown below. Working standards must be prepared on the day of use. Do not store diluted standards. Return the unused portion to of the standard concentrate to -20°C storage.

Pipets and/or pipet tips used to transfer the diluted standards must be of polypropylene or siliconized glass. Do not use those made of unsiliconized glass.
Table I - Suggested Dilution Scheme

<table>
<thead>
<tr>
<th>Tube</th>
<th>Concentration (pg/0.1 mL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.2 mL standard concentrate+ 1.8 mL Assay Buffer</td>
</tr>
<tr>
<td>B</td>
<td>0.2 mL of dilution A + 1.8 mL Assay Buffer</td>
</tr>
<tr>
<td>C</td>
<td>1.0 mL of dilution B + 1.0 mL Assay Buffer</td>
</tr>
<tr>
<td>D</td>
<td>1.0 mL of dilution C + 1.0 mL Assay Buffer</td>
</tr>
<tr>
<td>E</td>
<td>1.0 mL of dilution D + 1.5 mL Assay Buffer</td>
</tr>
<tr>
<td>F</td>
<td>1.0 mL of dilution E + 1.0 mL Assay Buffer</td>
</tr>
<tr>
<td>G</td>
<td>1.0 mL of dilution F + 1.0 mL Assay Buffer</td>
</tr>
<tr>
<td>H</td>
<td>1.0 mL of dilution G + 1.5 mL Assay Buffer</td>
</tr>
</tbody>
</table>

*This concentration represents actual mass added to the assay well. Dilution A should be used in the NSB wells. Dilutions B through H should be used for the standard curve.

C. Preparation of $[^{125}I]$ Working Tracer

For use in the assay, an appropriate aliquot of the tracer concentrate is diluted 1:20 (v:v) in assay buffer in a siliconized glass or polypropylene tube or vial. (For example, for a 20 well assay, dilute 0.1 mL of tracer concentrate with 2.0 mL assay buffer.) Dilute only enough tracer for use in each assay. Do not store diluted tracer. Return the unused portion of tracer concentrate to -20°C storage.

Pipets and/or pipet tips used to transfer the tracer solution must be of polypropylene or siliconized glass. Do not use those made of unsiliconized glass.

D. FlashAssay Protocol

1. Prepare all reagents according to directions.

2. Equilibrate all reagents to room temperature and mix before use.

3. Samples should be diluted in assay buffer. The extent of this dilution is sample dependent according to the amounts of PGE2 expected.

4. It is recommended that standards and samples be assayed in duplicate.

5. It is recommended that standard curves be run on each FlashPlate.

6. Pipet 100 µL of assay buffer into the zero standard wells.

7. Pipet 100 µL of each of the diluted standards (B - H) into the appropriate wells.
8. Pipet 100 µL of dilution (A) into the non-specific binding wells.

Note: The excess cold standard competes for all of the specific binding sites available to the [125I] tracer.

9. Pipet 100 µL of each diluted sample into the appropriate wells.
10. Pipet 100 µL of working tracer solution into each well set up for assay and also into two test tubes (12x75 mm) if total counts are desired.

Note: All participating wells should have 200 µL of liquid.

11. Place a plate cover on the plate. Attach the plate to a recommended microplate shaker and shake at the recommended setting as shown in Section VI.A. Incubate the plate, with shaking, overnight (18-24 hours) at 2-8°C.

Note: Be careful not to splash contents of wells while attaching plate to shaker.

12. After incubation, aspirate the entire contents of the wells to prevent re-equilibration of the assay at ambient temperature.

Note: Re-equilibration causes a shift of the standard curve to a less sensitive position and a reduction of the Bo signal. If the FlashPlate can be counted immediately (within 15 minutes) after removal from 2-8°C, aspiration of the wells is not necessary.

13. Count the plate on a 96 well microplate scintillation counter for one minute per well.

14. If all the wells of the FlashPlate have not been used, reseal the plate in the storage bag with desiccant and store at 2-8°C.

E. Alternative FlashAssay Protocol

This assay may be run at room temperature. This will result in a shift of the standard curve to a less sensitive position and a reduction in the Bo signal. The addition of a 200 pg/0.1 mL is recommended. See Table III for typical assay data.

VIII. CALCULATIONS

After counting has been completed, the concentration of prostaglandin E2 in the samples is determined by interpolation from the standard curve. The sample value determinations for diluted samples must be corrected for their respective dilutions. The following method is suggested.

A. If all wells have been counted for the same period of time, use the total accumulated counts; otherwise, correct all raw counts to counts per minute (CPM).

B. Average the counts for each set of duplicates.
C. Subtract the average NSB counts from each set of duplicates to determine the average net counts for each set.
D. Express the average net counts for each standard and sample as a percentage of the average counts for the net zero standard. (This is termed "normalized" percent bound or %B/Bo).

\[
%\text{B/Bo} = \frac{\text{Average Net Counts of Standard or Sample}}{\text{Average Net Counts of Zero Standard}} \times 100
\]

E. Using semi-logarithmic graph paper or equivalent software, plot % B/Bo for each standard versus the corresponding amounts of prostaglandin E2 added in picograms (pg). (See Figure 3 for a typical standard curve using the standard protocol.)

F. Determine pg prostaglandin E2 in each sample by interpolation from the standard curve. Since the standard curve is expressed as pg prostaglandin E2 added, sample values must then be corrected for aliquots, dilution, recovery, etc. to determine the original concentration in the sample.

**NOTE:** Any samples with concentrations which are above the range of the standard curve may be diluted with assay buffer and re-assayed. The values obtained are then multiplied by the appropriate dilution factor.

G. Kit performance data presented in this manual was generated with prostaglandin E2 standards which were prepared in assay buffer. The effect of other sample matrices upon the assay system must be determined by the investigator.
**Figure 3**

Typical Standard Curve
Do not use to calculate samples

![Typical Standard Curve Graph](image)

<table>
<thead>
<tr>
<th>Well</th>
<th>CPM</th>
<th>NET CPM (Mean CPM-NSB)</th>
<th>%B/Bo</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSB</td>
<td>341 / 325</td>
<td>2706</td>
<td>100</td>
</tr>
<tr>
<td>Bo Standard</td>
<td>3275 / 2988</td>
<td>2366</td>
<td>87</td>
</tr>
<tr>
<td>1 pg/0.1 mL</td>
<td>2747 / 2650</td>
<td>2076</td>
<td>77</td>
</tr>
<tr>
<td>2.5 pg</td>
<td>2429 / 2389</td>
<td>1677</td>
<td>62</td>
</tr>
<tr>
<td>5 pg</td>
<td>2124 / 1897</td>
<td>1381</td>
<td>51</td>
</tr>
<tr>
<td>10 pg</td>
<td>1755 / 1674</td>
<td>1093</td>
<td>33</td>
</tr>
<tr>
<td>25 pg</td>
<td>1222 / 1231</td>
<td>830</td>
<td>23</td>
</tr>
<tr>
<td>50 pg</td>
<td>982 / 942</td>
<td>409</td>
<td>15</td>
</tr>
<tr>
<td>100 pg</td>
<td>757 / 727</td>
<td>409</td>
<td></td>
</tr>
</tbody>
</table>

**Table II**

Typical data for Standard Protocol Assay at 4°C

<table>
<thead>
<tr>
<th>Well</th>
<th>CPM</th>
<th>NET CPM (Mean CPM-NSB)</th>
<th>%B/Bo</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSB</td>
<td>435 / 430</td>
<td>1406</td>
<td>100</td>
</tr>
<tr>
<td>Bo Standard</td>
<td>1874 / 1884</td>
<td>1295</td>
<td>92</td>
</tr>
<tr>
<td>5 pg/0.1 mL</td>
<td>1718 / 1738</td>
<td>1295</td>
<td></td>
</tr>
</tbody>
</table>
IX. PRECAUTIONS

A. Incubation conditions should be standardized for proper day to day internal quality control. This includes the strict control of the assay incubation temperature and the shaking process.

B. Pipetting must be performed reproducibly and accurately. Prostaglandin E2 has the tendency to adhere to many surfaces. To minimize assay interference from this “sticking” problem, use only polypropylene or siliconized glass pipets, or pipet tips when transferring diluted materials or incubating.

C. Since PGE2 can be converted to PGA2 as an artifact of sample handling, it is suggested that the samples be extracted as soon as possible after collection and that exposure to ambient temperatures be minimized.

D. This product has not been tested for use with any other methods other than those stated in this Instruction Manual.

E. WARNING: THIS PRODUCT CONTAINS A CHEMICAL KNOWN TO THE STATE OF CALIFORNIA TO CAUSE CANCER. (NOTE: [125I] TRACER)
X. PERFORMANCE CHARACTERISTICS

A. Specificity

The following compounds have been checked for cross-reactivity. The percentages are calculated at the 50% B/Bo point.

Table V

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Cross-Reactivity</th>
<th>Compound</th>
<th>% Cross-Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE₂</td>
<td>100</td>
<td>Arachidonic Acid</td>
<td>0.002</td>
</tr>
<tr>
<td>PGE₁</td>
<td>7</td>
<td>DHKPGF₂α</td>
<td>0.002</td>
</tr>
<tr>
<td>DHKPGE₂</td>
<td>0.008</td>
<td>15 KetoPGE₂</td>
<td>0.03</td>
</tr>
<tr>
<td>PGA₂</td>
<td>0.07</td>
<td>PGB₂</td>
<td>0.03</td>
</tr>
<tr>
<td>PGF₁α</td>
<td>0.2</td>
<td>6KPGF₁</td>
<td>0.16</td>
</tr>
<tr>
<td>Thromboxane B₂</td>
<td>0.003</td>
<td>Linoleic Acid</td>
<td>0.002</td>
</tr>
<tr>
<td>PGF₂α</td>
<td>0.2</td>
<td>PGD₂</td>
<td>0.02</td>
</tr>
</tbody>
</table>

B. Sensitivity

The mean and standard deviation were determined for 9 duplicate measurements of the zero standard binding in the standard protocol assay at 4°C. The sensitivity of the method, defined as the PGE₂ concentration corresponding to the mean cpm minus twice the standard deviation, is approximately 6.0 pg/mL.

C. Reproducibility

Within plate precision was determined by assaying multiple standard curves on a plate. Between plate precision was determined by averaging duplicate standard curves from several plates.

<table>
<thead>
<tr>
<th>Standard</th>
<th>n</th>
<th>Mean ± 1 S.D. cpm</th>
<th>Coeff of Var. (%)</th>
<th>Mean ± 1 S.D. cpm</th>
<th>Coeff of Var. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 pmol</td>
<td>12</td>
<td>3188 ± 208</td>
<td>2.8</td>
<td>0 pmol</td>
<td>3188 ± 185</td>
</tr>
<tr>
<td>1.0</td>
<td>12</td>
<td>2710 ± 164</td>
<td>4.5</td>
<td>1.0</td>
<td>2858 ± 129</td>
</tr>
<tr>
<td>2.5</td>
<td>12</td>
<td>2347 ± 183</td>
<td>3.5</td>
<td>2.5</td>
<td>2557 ± 102</td>
</tr>
<tr>
<td>5.0</td>
<td>12</td>
<td>2112 ± 73</td>
<td>4.6</td>
<td>5.0</td>
<td>2167 ± 88</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td>1714 ± 94</td>
<td>6.3</td>
<td>10</td>
<td>1818 ± 72</td>
</tr>
<tr>
<td>25</td>
<td>12</td>
<td>1276 ± 116</td>
<td>6.0</td>
<td>25</td>
<td>1303 ± 61</td>
</tr>
<tr>
<td>50</td>
<td>12</td>
<td>941 ± 45</td>
<td>6.8</td>
<td>50</td>
<td>953 ± 45</td>
</tr>
<tr>
<td>100</td>
<td>12</td>
<td>721 ± 49</td>
<td>6.8</td>
<td>100</td>
<td>712 ± 45</td>
</tr>
</tbody>
</table>
XI. REFERENCES


FlashPlate® is a registered trademark of Packard and is manufactured exclusively for NEN™ Life Science Products, Inc. under U.S. patent #4,626,513 and foreign equivalents.
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