# FP ONE-STEP REFERENCE KIT PROTOCOL

**Part # P3088**

**Lit. # 839-0480618 041604**

### A note For Beacon® 2000 One-Step FP Standardization Kit (PanVera® Part No. P2581) and Red (FP) Standardization Kit (PanVera® Part No. P2888) users:

As part of our continuing process improvement efforts, the three items in our Beacon® 2000 One-Step FP Standardization Kit (PanVera® Part No. P2581) have been reformulated to improve manufacturability while maintaining the same product performance specifications. We have changed the names of the components of the Red (FP) Standardization Kit (PanVera® Part No. P2888) but the Part Numbers and formulations remain the same. You can expect to receive the same performance with the new product formulation without necessary changes to existing equipment or experimental designs. As with all new lots of FP reference solutions, you should confirm your zeta settings for your specific instrument before use.

| Old Item | | New Item | |
|---|---|---|---|
| **Part #** | **Description** | **Part #** | **Description** |
| P2578 | Beacon® 2000 Low Polarization Standard | P3089 | Green Low Polarization Reference |
| P2579 | Beacon® 200 High Polarization Standard | P3090 | Green High Polarization Reference |
| P2580 | Beacon® 2000 BGG/Phosphate Buffer | P3091 | Green Polarization Reference Buffer |
| P2889 | Red Polarization Standard | P2889 | Red Low Polarization Reference |
| P2890 | Red Polarization Standard Buffer | P2890 | Red Polarization Reference Buffer |

Additionally, this protocol replaced Lit. # L0083 (PanVera® Part No. P2581) and Lit. # L0606 (PanVera® Part No. P2888). If you have any questions about this change, please contact Invitrogen's Technical Service at tech_service@invitrogen.com.

## 1.0 INTENDED USE

Many Fluorescence Polarization (FP) reading instruments require the use of a reference standard for internal calibration of the reader. The FP One Step Reference Kit contains reagents to use as green or red FP references in a single tube or a microplate high throughput screening (HTS) instrument. The green reagents can be used for readings using the following filters: 488 nm ± 5 nm for excitation and 535 nm ± 5 nm for emission (or equivalent). The red reagents can be used for readings using the following filters: 525 nm ± 5 nm for excitation and 590 nm ± 5 nm for emission (or equivalent).

## 2.0 DESCRIPTION

### 2.1 Materials Supplied

| Description | Composition | Amount | Part # |
|---|---|---|---|
| Green Polarization Reference Buffer | Proprietary Buffer (pH 7.4) | 15 mL | P3091 |
| Green Low Polarization Reference (Green LPR) | 1 nM fluorescein in Green Polarization Reference Buffer (pH 7.4) | 4 mL | P3089 |
| Green High Polarization Reference (Green HPR) | Proprietary in Green Polarization Reference Buffer (pH 7.4) | 4 mL | P3090 |
| Red Polarization Reference Buffer | Proprietary Buffer (pH 7.4) | 8 mL | P2890 |
| Red Low Polarization Reference (Red LPR) | 10 nM Rhodamine Derivative in Red Polarization Reference Buffer (pH 7.4) | 4 mL | P2889 |

### 2.2 Materials Required but Not Supplied

Fluorescence polarization instrument equipped with two sets of filters and appropriate mirror systems if needed.

Filter sets:

- For Green wavelength readings: 488 nm ± 5 nm excitation filter and 535 nm ± 5 nm emission filter (or equivalent)
- For Red wavelength readings: 525 nm ± 5 nm excitation filter and 590 nm ± 5 nm emission filter (or equivalent)

Pipetting devices for accurate delivery of required volumes.

Disposable 10 × 75 mm or 6 × 50 mm borosilicate test tubes, certified for use in the Beacon® 2000 Instrument or microplates suitable for use in FP HTS instruments.

### 3.0 Storage and Stability

All kit components should be stored at 20-30°C. Reagents are stable for 6 months after purchase.

*Note:* Fluorescence polarization is dependent upon temperature and viscosity of the sample solution. Allow all reagents to equilibrate to the chosen experimental temperature before performing polarization measurements.

## 4.0 GENERAL PROCEDURE

For detailed information regarding the operation of your fluorescence polarization system using molecules which excite and emit in the "green" or "red" ranges, please refer to your instrument's Operator's Manual.

### 4.1 Prepare the Reagents

| Tube/Well Designation | Green Filters | Red Filters |
|---|---|---|
| Blank | Green Polarization Reference Buffer (PanVera® Part No. P3091) | Red Polarization Reference Buffer (PanVera® Part No. P2890) |
| Low Polarization Reference | Green LPR (PanVera® Part No. P3089) | Red LPR* (PanVera® Part No. P2889) |
| High Polarization Reference | Green HPR (PanVera® Part No. P3090) | Not Applicable† |

* When using the Red LPR (10 nM) as an FP standard, if your fluorophore concentration is less than 5 nM, it may be necessary to dilute the Red LPR in the Red Polarization Reference Buffer to approximately the same intensity of your sample. This will optimize sample detection. (Be sure to record the concentration of your diluted Red LPR.)

† A High Polarization Reference is not generally required for Red filter based assays.

1. According to the above table, add reference reagents to designated tubes or wells. Use a volume equivalent to the sample tubes or wells.
2. Use multiple wells or multiple tubes as required by your instrument.

### 4.2 Run the Protocol

1. Using the specifications set for your instrument, run your samples to obtain an intensity and polarization value. The intensity reading can be used to approximate the concentration of your samples.

## 5.0 DETAILED BEACON® 2000 PROCEDURE

Please refer to the Beacon® 2000 Operator's Manual for detailed information regarding the operation of the Beacon® 2000 Analyzer.

### 5.1 Preparation

1. Turn on the Beacon® 2000 Analyzer. The instrument will perform a diagnostic procedure and produce a warning message if the RS-232 port or the printer are not online. Press **ENT** (Enter) to acknowledge any "RS-232" or "Printer not connected" warnings, as these devices must be brought online before you can send data to them. The instrument will then display the first item of the Main Menu "1) Run Protocol." Allow the instrument to warm up for at least 20 minutes before further use.
2. Turn to **Section 6.0** of this protocol and record your name, today's date, Beacon® 2000 FP One Step Reference Kit lot number, and Beacon® 2000 Analyzer equipment number in the spaces provided in the Data Record on **Section 6.0**.

### 5.2 Create a Beacon® 2000 Protocol

Protocol #2 on your Beacon® 2000 Analyzer has been preprogrammed for this assay. If you are using this protocol, or if you have already programmed the correct protocol, proceed to **Section 6.4**. Always confirm that the protocol printed on the thermal paper printout is the correct protocol. The general procedure is as follows:

1. Using the **UP** and **DWN** keys, toggle to the "Create Protocol" option and press the **ENT** key. The Beacon® 2000 Analyzer will report the number of open protocol numbers. Press **ENT** again.
2. For the "Protocol ID #", type "2" or any number between 2 and 99 that has not already been defined and press **ENT**.
3. Enter a 4-digit password using the numerical part of the instrument keypad and press **ENT**.

4. From the "Select Read Mode" menu, use the **UP** and **DWN** keys to select "Kinetic" and then press **ENT**. From the "Blanks?" menu, select **Yes**, then press **ENT** to input a default "Blank Delay" of 0 seconds.
5. To the "Initial Read?" prompt press **No**, then press **ENT** to input a default "Sample Delay" of 0 seconds.
6. At the " Number of Average Read Cycles" prompt, type "10". Then press **ENT**.
7. From the "Read Interval" prompt, press **ENT** twice to set the read interval to 0 minutes and 0 seconds. The read interval will actually be 14 or 24 seconds because the Beacon® 2000 Analyzer requires a minimum of 4 seconds plus 2 times the read cycle number to produce a measurement (refer to the instruction manual for a full description of minimum read time).
8. Press **ENT** to select the default "Set Temperature" setting and press **ENT** again to select the default temperature of 37˚C.
9. Press ENT two more times to select the default "Auto Range" and "Printer" options. You may review a hard copy of the protocol by pressing YES to the "Print Protocol" prompt. Your printout should look like this:

| PROTOCOL ID#2 | |
|---|---|
| Read Mode | => Kinetic |
| Blank Mode | => Single Blank |
| Blank Delay (sec) | => 0 |
| Sample Delay (sec) | => 0 |
| Average Read Cycles | => 10 |
| Initial Read | => No |
| Read Interval | => min:00 sec:00 |
| Range Control | => Auto Range |
| Temperature parameter | => Set Temp. |
| Single Point Temp (°C) | => 37 |
| Data Output assigned to | => Printer |

10. If you have created the protocol correctly, press YES, at the "Store New Protocol?" prompt and the protocol will be saved under the designated Protocol ID #.

## 5.3 Prepare the Reagents

1. Label three 6 × 50 mm borosilicate tubes "Blank", "Low", and "High".
2. Add 0.1 mL of Green Reference Buffer (Part # P3091) to the tube marked "Blank".
3. Add 0.1 mL of Low Polarization Reference (Part # P3089) to the tube marked "Low", and 0.1 mL of High Polarization Reference (Part # P3090) to the tube marked "High".

   ***Note:*** If you are using a Beacon® 2000 Analyzer with a 10 mm chamber, we recommend using minimum volumes of 500 µL, and using average read cycles of "5".

## 5.4 Run the Beacon® protocol

1. Press Run, type in the preprogrammed Protocol ID #, and press ENT.
2. The instrument will prompt you to "Insert Blank". The fluorescence intensity and polarization of the buffer will be subtracted from all subsequent measurements.
3. The instrument will prompt you to "Remove Blank Tube". Insert the tube marked "Low" when prompted to "Insert Sample". This protocol will take measurements every 14 or 24 seconds (depending on the tube size). Allow the Beacon® 2000 Analyzer to take 10 measurements, then press Stop on the Keypad. The Beacon® 2000 Analyzer will prompt you to "Remove Sample Tube".

   ***Note:*** Due to temperature variations in different laboratories, you may need to perform more than 10 readings to reach equilibrium. The last 5 data points should plateau nicely. If you do not see this, take more readings.

4. Record intensity and polarization values from readings 6-10 in the Data Record table on page 6. Readings 1-5 are not used because at this point the standard has not yet reached thermal equilibrium.
5. When prompted for "Another Run?", place your tube marked "High" in the chamber and press Yes. Repeat steps 1 through 4. When the Beacon® prompts you again for "Another Run?", press No. The instrument will then prompt "End of Reading".

## 6.0 DATA RECORD

| | |
|---|---|
| **User Name:** | |
| **Date:** | |
| **FP One Step Reference Kit (PanVera® Part No. P3088) Lot:** | |
| **Green Polarization Reference Buffer (PanVera® Part No. P3091) Lot:** | |
| **Green Low Polarization Reference (PanVera® Part No. P3089) Lot:** | |
| **Green High Polarization Reference (PanVera® Part No. P3090) Lot:** | |
| **Red Polarization Reference Buffer (PanVera® Part No. P2890) Lot:** | |
| **Red Low Polarization Reference (PanVera® Part No. P2889) Lot:** | |
| **Instrument Serial Number:** | |

| **Low Polarization Reference Data** | | | |
|---|---|---|---|
| **Reading Number** | **Fluorescence Intensity (Tot Int)** | | **Polarization (mP)** |
| **6** | | | |
| **7** | | | |
| **8** | | | |
| **9** | | | |
| **10** | | | |
| | **Actual Value** | **Specification** | **Pass/Fail?** |
| **Blank Int** | | | |
| **Mean Tot Int** | | | |
| **Mean mP** | | 20 mP 5 | |
| **mP Std Dev** | | 2 | |
| Mean Tot Int/Blank Int | | 45 | |

| **High Polarization Reference Data** | | | |
|---|---|---|---|
| **Reading Number** | **Fluorescence Intensity (Tot Int)** | | **Polarization (mP)** |
| **6** | | | |
| **7** | | | |
| **8** | | | |
| **9** | | | |
| **10** | | | |
| | **Actual Value** | **Specification** | **Pass/Fail?** |
| **Mean mP** | | > 340 mP | |
| **mP Std Dev** | | 8 | |

Tape the Beacon® 2000 thermal paper printout on the back of this page.
This instrument meets all quality control specifications:

**Signed** **Date**

The performance of this product is guaranteed for six months from the date of purchase if stored and handled properly.
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