INSTRUCTIONS

QuantaBlu™ Fluorogenic Peroxidase Substrate Kits

15169 15162

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Number	Description
15169	**QuantaBlu Fluorogenic Peroxidase Substrate Kit**, for stopped, nonstopped and/or kinetic assays **Kit Contents:** **QuantaBlu Substrate Solution,** 250mL **QuantaBlu Stable Peroxide Solution,** 30mL **QuantaBlu Stop Solution,** 275mL
15162	**QuantaBlu NS/K Fluorogenic Substrate Kit**, for nonstopped and/or kinetic assays **Kit Contents:** **QuantaBlu Substrate Solution,** 250mL **QuantaBlu Stable Peroxide Solution,** 30mL

Note: The excitation and emission maxima for QuantaBlu Fluorogenic Peroxide Substrates are 325nm and 420nm, respectively. Wavelengths between 315 and 340 nm for excitation and 370 and 470nm for emission can also be used.

Storage: Upon receipt store at 4°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific QuantaBlu Fluorogenic Peroxidase Substrate is a soluble fluorogenic (chemifluorescent) substrate for detection of peroxidase activity. The QuantaBlu Working Solution in the presence of active peroxidase produces a blue fluorescent product that can be quantitated by fluorometry in microplates or cuvettes. Fluorometric-based detection has a large dynamic range, which overcomes the limitations of using spectrophotometry to measure colorimetric substrates. The blue fluorescent product is not sensitive to light and does not photobleach.

The QuantaBlu Fluorogenic Peroxidase Substrate is stable, highly sensitive, produces high signal-to-noise ratios and allows for a broad dynamic detection range of peroxidase activity. This substrate is also flexible and can be used for stopped, nonstopped and kinetic assays. Incubation times for stopped and nonstopped assays can be performed between 5 and 90 minutes at either room temperature (RT) or 37°C. The QuantaBlu NS/K Substrate can be integrated into robotic-based assays and is designed for assays that do not need to be stopped

Important Product Information

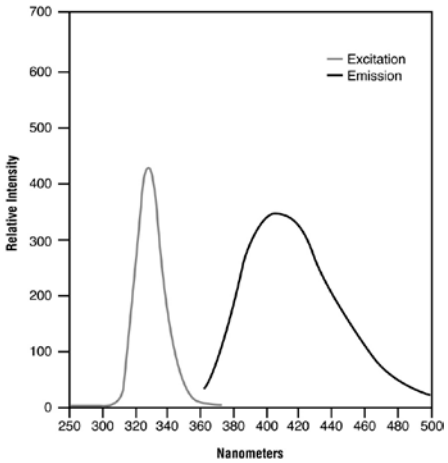

Figure 1. Exitation/Emission spectra of Thermo Scientific QuantaBlu Substrate.

- QuantaBlu Fluorogenic Peroxidase Substrate is detected by fluorometric methods using appropriate excitation and emission settings (see Figure 1). Quantitation does not require filters that precisely match the excitation/emission maxima, but a non-overlapping filter set that has a bandpass that includes the excitation/emission spectra is required.
- In a nonstopped assay, this coloration can act as a fluorescence-quenching agent and impart a strong tailing hook to a standard curve. However, when the stop solution is added, the brown color is immediately removed and standard curves are not negatively affected.
- Instrumentation-specific settings will affect the performance of the fluorogenic substrate. High gain settings will increase signal intensity. Increasing the photomultiplier tube voltage will also increase signal intensity. Increase the integration times and/or flash count to enhance precision. The bandpass range of the excitation and/or emission filter may be increased or decreased to achieve any requirement for signal intensity. Fluorometric units are typically defined as relative fluorescence units (RFU) because the integrated signal is dependent on instrument settings. Consult the fluorometer's user manual for specific instrument capabilities and settings.
- Fluorometric assays typically use white or black opaque microplates. White plates typically offer greater signal detection, as well as higher background than black plates. A variety of other plate types, such as gray plates or opaque plates with transparent bottoms are also useful with this substrate. Traditional transparent microplates might help in spot-check assays using spatially distant wells or in qualitative assays, but signal-to-noise ratios are very low.
- The QuantaBlu Fluorogenic Peroxidase Substrate is highly sensitive. Avoid accidental contamination of the substrate with peroxidase during pipetting of the working solution into the wells.
- Problems with high background will require optimization of assay components, such as antibody, conjugate and blocking buffer. Opaque black plates often result in less background than opaque white plates.
- Shaking the plate briefly after adding the QuantaBlu Stop Solution is not required but may decrease the standard deviation because the well contents become more homogenous. Furthermore, increasing the flash count and/or integration time on the fluorometer might improve precision of replicates.

Example Microplate Protocol

The following protocol is an example application for this product. Specific applications will require optimization.

A. Additional Materials Required

- Carbonate/bicarbonate buffer, pH 9.4 (Thermo Scientific BupH Carbonate-Bicarbonate Buffer Packs, Product No. 28382)
- Tween®-20 Detergent (Thermo Scientific Surfact-Amps 20, Product No. 28320)
- Blocking Buffer**:** Thermo Scientific SuperBlock (PBS) Blocking Buffer (Product No. 37515) or SuperBlock® (TBS) Blocking Buffer (Product No. 37535) with Tween-20 Detergent at a final concentration of 0.05%

  Note: Because no blocking reagent is optimal for all systems, empirical testing is essential to determine the appropriate blocking buffer for each system. Determining the proper blocking buffer can help increase sensitivity and prevent nonspecific signal caused by cross-reactivity with the blocking reagent.
- Wash Buffer: BupH™ Tris Buffered Saline Packs (Product No. 28379; contains 0.25M Tris, 0.15M NaCl, pH 7.2) or BupH Phosphate Buffered Saline Packs (Product No. 28372; contains 0.1M phosphate, 0.15M NaCl, pH 7.2)

B. Material Preparation

QuantaBlu Working Solution (WS)	Mix 9 parts of QuantaBlu Substrate Solution to 1 part of QuantaBlu Stable Peroxide Solution. The WS is stable for 24 hours at RT and no protection from light is required. To reduce variability, equilibrate the WS to RT before adding to the wells.
Capture Antibody	Dilute capture antibody to 5-10µg/mL in carbonate/bicarbonate buffer
Primary Antibody	Dilute primary antibody to 0.05-0.1µg/mL in wash buffer
HRP Conjugate	Dilute conjugate to 25-50ng/mL in wash buffer

C. Procedure

1. Add 50-100µL of the capture antibody to each well and incubate for 1 hour at room temperature (RT).
2. Invert plate to empty wells. Blot plate three times on a stack of paper towels.
3. Add 300µL Blocking Buffer to wells and incubate for 1 hour at RT.
4. Invert plate to empty wells. Blot plate three times on a stack of paper towels. Add antigen and incubate for 1 hour at RT.
5. Empty wells and wash three times for 5 minutes each on shaking platform in 200µL of wash buffer that contains 0.05% Tween-20 Detergent.
6. Add 50-100µL primary antibody to each well and incubate for 1 hour at RT.
7. Invert plate to empty wells. Wash as indicated in step 5. Blot plate three times on a stack of paper towels.
8. Add 50-100µL of the HRP conjugate to each well. Incubate for 1 hour at RT.
9. Invert plate to empty wells. Wash as indicated in step 5. Blot plate three times on a stack of paper towels.
10. Add 100µL of QuantaBlu WS to each well and incubate for 1.5-90 minutes at RT or 37°C.

    Note: Primary antibodies that are directly HRP-conjugated will require further optimization for appropriate concentrations.

    Note: For prolonged incubation times or incubation at elevated temperatures, cover the plate with sealing tape (Product No. 15036) to prevent sample evaporation.

11. Stop peroxidase activity by adding 100µL of QuantaBlu Stop Solution; the enzymatic activity is immediately stopped and incubation is not required.

    Note: A quick assay progress can be detected by using a hand-held long wave UV light source. Peroxidase activity will appear as strong blue fluorescence. Preliminary plate evaluations may also be performed without stopping because the substrate does not photobleach and assay progress is not negatively affected by exposure to light. At high peroxidase concentrations or with prolonged reaction times, the substrate appears as rosy-salmon to brown.

12. Measure relative fluorescence units (RFU) of each well. The excitation and emission maxima for QuantaBlu Substrate are 325nm and 420nm, respectively. Wavelengths between 315 and 340nm for excitation and 370 and 470nm for emission also can be used for detection.

Related Thermo Scientific Products

15159	**QuantaRed® Enhanced Chemifluorescent Substrate Kit**
15520	**High Sensitivity Streptavidin Coated Plates,** 5/pkg
15530	**High Sensitivity NeutraAvidin Coated Plates,** 5/pkg
15119	**Streptavidin Coated Black 96-well plates with SuperBlock Blocking Buffer,** 5/pkg
15407	**Streptavidin Coated Black 384-well plates with SuperBlock Blocking Buffer,** 5/pkg
15118	**Streptavidin Coated White 96-well plates with SuperBlock Blocking Buffer,** 5/pkg
15406	**Streptavidin Coated White 384-well plates with SuperBlock Blocking Buffer,** 5/pkg
29139	**Biotinylated Horseradish Peroxidase,** 5mg
15075	**Reagent Reservoirs,** 200/pkg
15082	**Microtube Racked System,** 960 tubes
15036	**Sealing Tape for 96-Well Plates,** 100/pkg

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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