QuantaBlu™ Fluorogenic Peroxidase Substrate Kits

Catalog Numbers 15169, 15162
Pub. No. MAN0011341 Rev. B

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. SDSs are available from thermofisher.com/support.

Product description

The Thermo Scientific™ QuantaBlu™ Fluorogenic Peroxidase Substrate is a soluble chemifluorescent substrate designed for detecting peroxidase activity. When the QuantaBlu™ Working Solution encounters active peroxidase, it produces a blue fluorescent product that can be quantified using fluorometry in microplates or cuvettes. This fluorometric detection method offers a large dynamic range, overcoming the limitations of spectrophotometric colorimetric measurements. Additionally, the blue fluorescent product is stable, resistant to light, and does not photobleach, ensuring reliable and consistent results.

Features of QuantaBlu™ Fluorogenic Peroxidase Substrate Kits include:

- **Stability:** The QuantaBlu™ Fluorogenic Peroxidase Substrate is highly stable and resistant to photobleaching.
- **Flexibility:** The substrate is suitable for stopped, non-stopped, and kinetic assays.
- **Efficient and reproducible:** Provides highly sensitive detection of peroxidase activity.
- **Sensitivity:** Obtain results with as few as 10,000 cells.
- **High signal-to-noise ratios:** Produces high signal-to-noise ratios for accurate measurements.
- **Broad dynamic detection range:** Allows for a broad dynamic range in detecting peroxidase activity.
- **Versatile incubation times:** Incubation times for stopped and non-stopped assays can range from 5 to 90 minutes at room temperature (RT) or 37°C.
- **Robotic integration:** The QuantaBlu™ NS/K Substrate can be integrated into robotic-based assays.
- **Non-stop assay design:** Specifically designed for assays that do not need to be stopped.

Contents and storage

Table 1 QuantaBlu™ Fluorogenic Peroxidase Substrate Kits (Cat. No. 15169 and 15162)

Cat. No.	Item	Capacity	Storage
QuantaBlu™ Fluorogenic Peroxidase Substrate Kit for stopped, non-stopped and/or kinetic assays			
15169	QuantaBlu Substrate Solution	250 mL	Store at 4°C upon receipt. The product is shipped at ambient temperature.
	QuantaBlu Stable Peroxide Solution	30 mL	
	QuantaBlu Stop Solution	250 mL	
QuantaBlu™ NS/K Fluorogenic Substrate Kit for non-stopped and/or kinetic assays			
15162	QuantaBlu Substrate Solution	250 mL	Store at 4°C upon receipt. The product is shipped at ambient temperature.
	QuantaBlu Stable Peroxide Solution	30 mL	
Note: The QuantaBlu™ Fluorogenic Peroxidase Substrate has excitation and emission maxima at 325 nm and 420 nm, respectively. Additionally, excitation wavelengths between 315 nm and 340 nm and emission wavelengths between 370 nm and 470 nm are also suitable.			

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Cat. No.
Thermo Scientific™ Ready to use, Wash Buffer Stock Solutions	
TBS Tween™ 20 Buffer	28360
PBS Tween™ 20 Buffer	28352
Thermo Scientific™ Blocking Buffers	
SuperBlock™ (PBS) Blocking Buffer	37515
SuperBlock™ (TBS) Blocking Buffer	37535
Thermo Scientific™ Buffers	
Dry-blend Buffered Packs (Carbonate-Bicarbonate Buffer Packs)	28382
Dry-blend Buffered Packs (Tris Buffered Saline Packs), For use with Tween™-20 Surfact-Amps™ Detergent Solution	28379
Dry-blend Buffered Packs (Phosphate Buffered Saline–PBS), For use with Tween™-20 Surfact-Amps™ Detergent Solution	28372
Tween™-20 Surfact-Amps™ Detergent Solution	28320

Procedural guidelines

- QuantaBlu™ Fluorogenic Peroxidase Substrate can be detected using fluorometric methods with suitable excitation and emission settings (See figure 1 on page 3). Precise matching of excitation/emission maxima is not necessary; however, a non-overlapping filter set with a bandpass covering the excitation/emission spectra is required.
- In non-stopped assays, the coloration can quench fluorescence and distort the standard curve. Adding the Stop Solution eliminates the brown color, ensuring the standard curves remain unaffected.
- Instrumentation settings can affect the performance of the fluorogenic substrate. High gain settings and increased photomultiplier tube voltage boost signal intensity. Enhancing integration times and flash count improves precision. Adjusting the bandpass range of the excitation/emission filter can modify signal intensity. Fluorometric units are usually expressed as relative fluorescence units (RFU) due to dependency on instrument settings. Refer to the fluorometer's user manual for specific capabilities and settings.
- Fluorometric assays commonly use white or black opaque microplates.White plates offer higher signal detection but also produce more background noise compared to black plates. Additionally gray plates and opaque plates with clear bottoms are also effective. Transparent microplates can be useful for spot-check or qualitative assays, though they offer low signal-to-noise ratios
- The QuantaBlu™ Fluorogenic Peroxidase Substrate is highly sensitive. Avoid contaminating the substrate with peroxidase during pipetting of the Working Solution into the wells.
- To address high background issues, optimize assay components like antibody, conjugate, and blocking buffer. Using opaque black plates typically results in lower background compared to opaque white plates.
- Briefly shaking the plate after adding QuantaBlu™ Stop Solution can reduce standard deviation by homogenizing the well contents. Increasing the flash count and/or integration time on the fluorometer can also enhance replicate precision.

Reagent preparation

Reagent	Preparation
QuantaBlu™ Working Solution (WS)	Mix 9 parts of QuantaBlu™ Substrate Solution to 1 part of QuantaBlu™ Stable Peroxide Solution. **Note:** The Working Solution (WS) remains stable for 24 hours at room temperature and does not require light protection. To minimize variability, ensure the WS is equilibrated to room temperature before adding it to the wells.
Capture Antibody	Dilute Capture Antibody to 5–10 µg/mL in Carbonate-Bicarbonate Buffer.
Primary Antibody	Dilute Primary Antibody to 0.05–0.1 µg/mL in Wash Buffer.
HRP Conjugate	Dilute Conjugate to 25–50 ng/mL in Wash Buffer.
Wash Buffer	TBS or PBS with 0.05% Tween™ 20.

Microplate protocol

1. Add 50–100 µL of the Capture Antibody to each well and incubate for 1 hour at room temperature (RT).
2. Invert the plate to empty the wells. Blot the plate 3 times on a stack of paper towels.
3. Add 300 µL Blocking Buffer to the wells and incubate for 1 hour at RT.
4. Invert the plate to empty the wells. Blot the plate 3 times on a stack of paper towels. Add the antigen and incubate for 1 hour at RT.
5. Empty the wells and wash three times for 5 minutes each on a shaking platform in 200 µL of Wash Buffer.

   Note: The Wash Buffer contains 0.05% Tween™ 20 Detergent.
6. Add 50–100 µL of the Primary Antibody to each well and incubate for 1 hour at RT.
7. Invert the plate to empty the wells. Wash as indicated in step 5. Blot the plate 3 times on a stack of paper towels.
8. Add 50–100 µL of the HRP Conjugate to each well and incubate for 1 hour at RT.
9. Invert the plate to empty the wells. Wash as indicated in step 5. Blot the plate 3 times on a stack of paper towels.
10. Add 100 µL of QuantaBlu™ WS to each well and incubate for 90 minutes at RT or 37°C

    Note:
    - Optimize Primary Antibodies that are directly HRP-Conjugated for appropriate concentrations.
    - Cover the plate with Sealing Tape (Cat. No. 15036) to prevent sample evaporation during prolonged incubation times or incubation at elevated temperatures.
11. Stop peroxidase activity by adding 100 µL of QuantaBlu™ Stop Solution; the enzymatic activity stops immediately and incubation is not required.

    Note: Detect quick assay progress using a hand-held long wave UV light source. Peroxidase activity appears as strong blue fluorescence. Perform preliminary plate evaluations 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 rosy-salmon to brown.
12. Measure relative fluorescence units (RFU) of each well. Use excitation and emission maxima of 325 nm and 420 nm, respectively. Wavelengths between 315 nm and 340 nm for excitation and 370 nm and 470 nm for emission can also be used for detection.

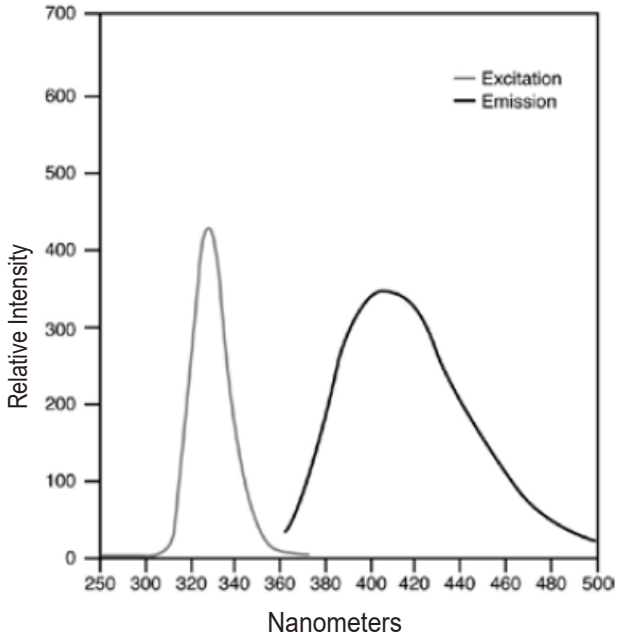

Figure 1 Image depicting the excitation and emission wavelengths

Related products

Product	Cat. No.	Amount
QuantaRed™ Enhanced Chemifluorescent HRP Substrate Kit	15159	110 mL
Pierce™ NeutrAvidin™ Coated High Sensitivity Plates, Clear, 8-Well Strip	15530	5 plates
Pierce™ Streptavidin Coated Black 96-well Plates with SuperBlock™ Buffer	15119	5 plates
Pierce™ Streptavidin Coated Black 384-well Plates with SuperBlock™ Buffer	15407	5 plates
Pierce™ Streptavidin Coated White 96-well Plates with SuperBlock™ Buffer	15118	5 plates
Pierce™ Horseradish Peroxidase, Biotinylated	29139	5 mg
ELISA Reagent Reservoir	15075	200 units
Sealing Tape for 96-Well Plates	15036	100 sheets

Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0011341 B

Revision	Date	Description
B	18 September 2025	• Updated template for branding and legal boilerplates. • Volume changed from 275 mL to 250 mL for QuantaBlu™ Stop Solution in the Contents and Storage table. • Version numbering was also changed to new format and reset to B in conformance with internal document control procedures.
A	17 October 2015	New document for the QuantaBlu™ Fluorogenic Peroxidase Substrate Kits.