Amplex® Red/UltraRed Stop Reagent

Introduction

Amplex® UltraRed and Amplex® Red assay kits provide sensitive biomolecular assays based on hydrogen peroxide–generating enzyme systems linked to peroxidase–mediated oxidation of the fluorogenic Amplex® UltraRed or Amplex® Red substrates.[1,2,3] Typically, detection reactions are performed in microplate wells and are initiated by adding the fluorogenic Amplex® UltraRed or Amplex® Red substrate, resulting in a continuous fluorescence increase that proceeds for 30 minutes or more. Ultimately, unknown analyte concentrations are determined by referencing fluorescence intensities measured at a certain time point during the reaction to parallel measurements at the same time point on standard samples of known concentration. Clearly, it is quite critical to ensure that the timing of the standard and unknown sample measurements is the same. The Amplex® Red/UltraRed stop reagent provides convenience and control by allowing the fluorescence signal-generating reaction to be terminated at a user-determined time point (Figure 1). After addition of the stop reagent, the fluorescence signal remains stable for at least 3 hours. The Amplex® Red/UltraRed stop reagent is designed for use in conjunction with both the Amplex® Red and Amplex® UltraRed fluorogenic substrates and assay kits (Figure 2). It is designed to terminate reactions containing up to 0.1 units/mL of horseradish peroxidase (HRP) and 5 µM H_2O_2. In principle, the Amplex® Red/UltraRed stop reagent should also be effective in other HRP-coupled nonradioactive detection systems, although we have not yet validated its performance in such systems.

Materials

- **Amplex® Red/UltraRed stop reagent**, 5 vials. Each vial contains sufficient material to terminate 100 detection reactions as defined in the protocol described below.

Materials required but not provided

- **Ethanol** and **water** for reconstitution
- **Vial or pipettor reservoir** for dilution of stop solution (note A).

Storage and Handling

Store refrigerated at ≤6°C until required for use. If frozen, avoid freeze-thaw cycles. Desiccation is recommended but not essential.

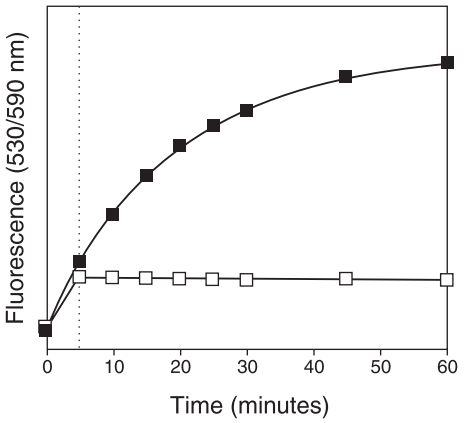

Figure 1. Application of the Amplex® Red/UltraRed stop reagent to control H_2O_2/peroxidase-coupled detection reactions. Two parallel reactions containing 0.5 mU/mL horseradish peroxidase in 50 mM sodium phosphate buffer, pH 7.4 were initiated by addition of 50 µM Amplex® Red reagent + 1 mM H_2O_2. Reaction progress was monitored by detection of the fluorescent product resorufin at 37°C in a fluorescence microplate reader using excitation at 530 ± 12.5 nm and fluorescence detection at 590 ± 17.5 nm. After five minutes (⋅), one of the reactions (□) was terminated by addition of Amplex® Red/UltraRed stop reagent. The fluorescence signal in the stopped reaction remained at the constant level shown for 3 hours (data not shown).

Protocol

1.1. Reconstitute one vial of Amplex® Red/UltraRed stop reagent by adding 1.45 mL of ethanol. Vortex or agitate briefly. Transfer 1.25 mL of this solution to user-supplied vial or pipettor reservoir (note A) and dilute with an equal volume (1.25 mL) of water, giving a total of 2.5 mL of stop solution. This amount is sufficient to stop 100 detection reactions of 100 µL each (note B). After reconstitution, the stop reagent is stable for approximately one month stored at 2–6°C in the dark. The stop solution should be colorless. Appearance of amber coloration is indicative of decomposition.

1.2. At the desired stopping time point in the Amplex® Red or Amplex® UltraRed microplate assay, add 20 µL of Amplex® Red/UltraRed stop reagent solution per 100 µL sample in each microplate well (note B). The stop reagent should be added to all wells, including any reagent controls containing Amplex® Red or Amplex® UltraRed reagents but no HRP (note C). The time-dependent fluorescence signal increase will cease immediately and the fluorescence signal level should remain stable for at least 3 hours.

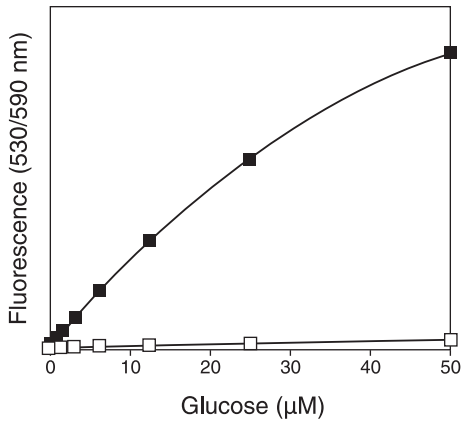

Figure 2. Application of the Amplex® Red/UltraRed stop reagent in the standard Amplex® Red Glucose/Glucose Oxidase Assay protocol. Reactions containing 50 µM Amplex® Red reagent, 0.1 U/mL HRP, 1 U/mL glucose oxidase and the indicated amounts of glucose in 50 mM sodium phosphate buffer, pH 7.4, were analyzed in a fluorescence microplate reader (excitation at 530 ± 12.5 nm, fluorescence detection at 590 ± 17.5 nm) after 30 minutes incubation at room temperature (■). A parallel set of reactions (□) contained 20 µL of Amplex® Red/UltraRed stop reagent per 100 µL sample added at the initiation of the reactions. The data demonstrate the complete inhibition of signal development by the stop reagent. Note that in coupled assay applications, the stop reagent would usually be added after allowing some time for signal development (user-defined, typically 10–60 minutes), not at the initiation point as in this example.

Notes

[A] Capacity of ≥3 mL is required.

[B] This protocol is based on a reaction volume of 100 µL, as typically used in 96-well microplates. For other reaction volumes, adjust the addition of Amplex® Red/UltraRed stop reagent solution proportionally (e.g. add 8 µL to a 40 µL reaction volume).

[C] Addition to controls is required to take account of the ~17% dilution of the samples upon addition of the stop solution and also to compensate for the fluorescence quenching effect (typically <5%) of the Amplex® Red/UltraRed stop reagent stop reagent on the Amplex® Red and Amplex® UltraRed reagent oxidation products.

References

1. Anal Biochem 253, 162-168 (1997); **2.** Anal Biochem 305, 118-119 (2002); **3.** J Biochem Biophys Methods 38, 43-52 (1999).

Product List *Current prices may be obtained from our website or from our Customer Service Department.*

Cat #	Product Name	Unit Size
A33855	Amplex® Red/UltraRed stop reagent *500 tests*	1 set

Contact Information

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Please visit our website—**probes.invitrogen.com**—for the most up-to-date information.

Molecular Probes, Inc.
29851 Willow Creek Road, Eugene, OR 97402
Phone: (541) 465-8300 • Fax: (541) 335-0504

Customer Service: 6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338 • Fax: (541) 335-0305 • probesorder@invitrogen.com

Toll-Free Ordering for USA:
Order Phone: (800) 438-2209 • Order Fax: (800) 438-0228

Technical Service: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353 • Toll-Free (800) 438-2209
Fax: (541) 335-0238 • probestech@invitrogen.com

Invitrogen European Headquarters
Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100 • Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Limited Use Label License
For research use only. Not intended for any animal or human therapeutic or diagnostic use. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402. Tel: (541) 465-8300. Fax: (541) 335-0504.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.