Amplite™ Human Serum Albumin (HSA) Site II Binding Assay Kit

Catalog number: 25402
Unit size: 200 Tests

Component	Storage	Amount
Component A: HSA Blue™ S2	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: HSA Assay Buffer	Freeze (< -15 °C)	1 vial (30 mL)
Component C: Human Serum Albumin solution (HSA)	Freeze (< -15 °C)	1 vial (500 µL)
Component D: DMSO	Freeze (< -15 °C)	1 vial (100 µL)

OVERVIEW

Human serum albumin (HSA) is one of the most important carriers for acidic drugs in human plasma and has been shown to bind a large number of different compounds in a reversible manner. Several different ligand binding sites have been identified for HSA. Among them, Site II has been identified as one of major drug binding sites. Amplite™ Human Serum Albumin (HSA) Site II Binding Assay Kit is a fluorescence-based high throughput assay to determine the small molecule binding towards HSA. This assay is based on a novel fluorescent probe, HSA Blue™ S2. It has been characterized to bind to the site 2 of HSA with unique spectroscopic and binding properties. HSA Blue™ S2 displays a large fluorescence intensity difference between the protein-bound and protein-unbound state. The competition of small molecules for HSA binding in the presence of HSA Blue™ S2 results in low fluorescence intensities. This assay can be used as a high throughput screen tool to determine total binding to HSA at Site II.

AT A GLANCE

Protocol Summary

1. Add HSA working solution (50 µL) and HSA Blue™ S2 working solution (50 µL) to the wells
2. Add test drugs (50 µL) with various concentrations to respective wells
3. Incubate for 15 to 45 minutes at RT
4. Measure response with fluorescence microplate reader at Ex/Em = 365/480 nm (Cutoff = 435 nm)

Important
Bring all the kit components at room temperature before starting the experiment.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

HSA Blue™ S2 stock solution
Add 100 µL DMSO (Component D) into HSA Blue™ S2 (Component A) and mix well.

Note Store the unused HSA Blue™ S2 stock solution at -20 °C in single use aliquots to avoid freeze thaw cycles.

PREPARATION OF WORKING SOLUTION

1. HSA Blue™ S2 working solution
Add 50 µL HSA Blue™ S2 stock solution into 5 mL of HSA Assay Buffer (Component B) and mix well.

Note HSA Blue™ S2 working solution should not be stored and should be used promptly.

Note 5 mL HSA Blue™ S2 working solution is enough for one 96-well plate.

2. HSA working solution
Add 250 µL HSA solution (Component C) into 5 mL of HSA Assay Buffer (Component B) and mix well.

Note 5 mL HSA working solution is enough for one 96-well plate.

3. Test drugs working solution
Dilute drugs stock solution to the desired concentrations in 3X working solutions using HSA Assay Buffer (Component B).

Note For the protocol mentioned here, suggested volume for the one well is 50 µL.

SAMPLE EXPERIMENTAL PROTOCOL

The following protocol can be used as a guideline and should be optimized according to the needs.

1. Add 50 µL of HSA Blue™ S2 working solution in wells.
2. Add 50 µL of HSA working solution in wells.
3. Add 50 µL of drugs working solution to their respective wells. (Total volume = 150 µL/well).
4. Incubate the samples for 30 minutes at RT.
5. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 365/480 nm (Cutoff = 435 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

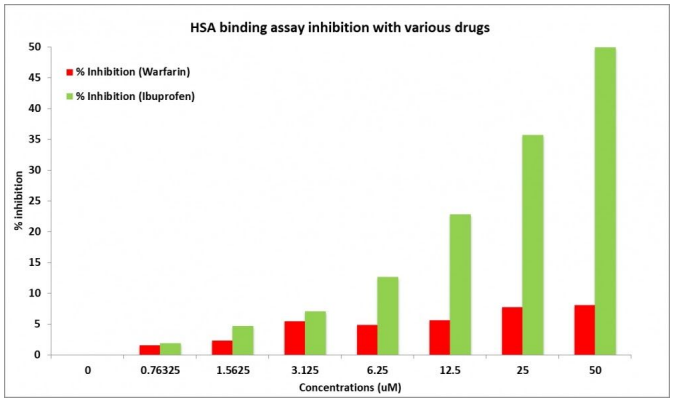

Figure 1. Response of Warfarin (Site-1 drug) and Ibuprofen (Site-2 drug) was measured using Amplite™ Human Serum Albumin (HSA) Site II Binding Assay Kit. The response was acquired using Spectra Max Gemini XS (Molecular devices) with Ex/Em = 365/480 nm with cutoff = 435 nm.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the

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