

Human Serum Albumin Binding Assay Kit

Item No. 502941

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. After opening the kit, store individual components as stated below.

Item Number	Item	Quantity/Size	Storage Temperature
401198	HSA TSA Buffer	1 vial/12 ml	RT
401199	TSA Probe	1 vial/300 µl	-20°C
401200	TSA Human Serum Albumin	1 vial/85 µl	-20°C
401201	HSA TSA Positive Control	1 vial/55 µl	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's Human Serum Albumin Binding Assay Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

It is recommended to take appropriate precautions when using the kit reagents as some may be harmful. Please wear appropriate personal protective equipment (*e.g.* safety glasses, gloves, and lab coat) when using these materials. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A quantitative PCR (qPCR) instrument capable of measuring fluorescence in the SYBR® Green channel or with excitation and emission wavelengths of 468 and 507 nm, respectively
2. A compatible (optically clear, capped/sealed) strip tube or qPCR plate
3. Adjustable pipettes; multichannel or repeating pipettor recommended
4. A source of pure water; glass-distilled water is acceptable. *NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000).*
5. Materials used for **Sample Preparation** (see page 10)

INTRODUCTION

Background

Human serum albumin (HSA) is a negative acute phase reactant and the most abundant protein in human plasma.[1] It is involved in many functions, such as the regulation of plasma osmotic pressure, extracellular antioxidant defense, and the binding and transport of various endogenous and exogenous compounds, including pharmaceuticals, environmental toxicants, and other xenobiotics.[1-3] Endogenous ligands of HSA include fatty acids, which are the primary physiological ligands, thyroxine, and metal ions, as well as larger molecules such as peptides and proteins.[1] HSA predominantly binds to acidic and neutral drugs in a reversible manner, which greatly influences their pharmacokinetics and pharmacodynamics.[1,4]

About This Assay

Cayman's HSA Binding Assay Kit provides a fluorometric thermal shift assay (TSA) method for measuring analyte binding to HSA. Probe fluorescence can be quantified at excitation and emission wavelengths of 468 and 507 nm, respectively, or the SYBR® Green channel of a qPCR instrument. This method detects increases in protein stabilization that occur with compound binding *via* measurement of the protein melting temperature (T_m). The probe used in this assay does not affect the stability of HSA, resulting in very consistent melting profiles and drug-induced thermal shifts. This assay is validated for evaluation of compound binding to well-characterized drug-binding sites, such as Sudlow's Sites I and II.

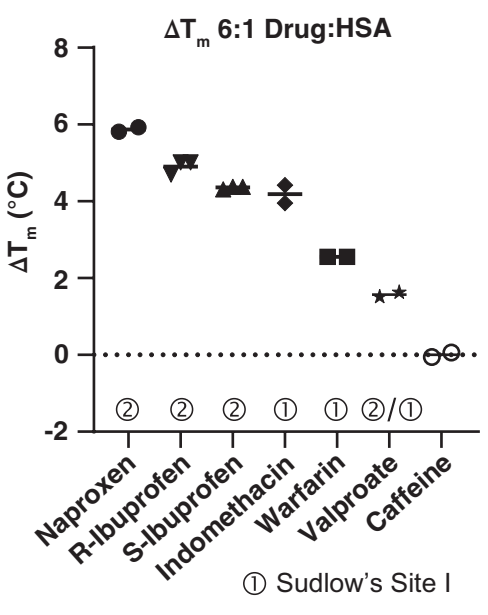


Figure 1. HSA T_m shifts for known Sudlow's Site-binding drugs and a negative control. All T_m shifts represented are at 60 µM drug treatments, or a 6:1 ratio of drug:HSA.

PRE-ASSAY PREPARATION

Reagent Preparation

NOTE: All reagents should be equilibrated to room temperature prior to starting the assay.

1. **HSA TSA Buffer (2X) – (Item No. 401198)**

   This vial contains 12 ml of HSA TSA Buffer (2X). Let the assay buffer warm to room temperature prior to use. The assay buffer will be stable for six months when stored at room temperature.

2. **TSA Probe – (Item No. 401199)**

   This vial contains 300 µl of the thermal shift assay fluorescent probe in water. It is ready to use as supplied. The unopened vial should be thawed at room temperature, protected from light. One vial contains a sufficient volume to assay 96 wells. The probe will be stable for six months when stored protected from light at room temperature.

3. **TSA Human Serum Albumin – (Item No. 401200)**

   This vial contains 85 µl of TSA Human Serum Albumin in water. It is ready to use as supplied. One vial is a sufficient volume to assay 96 wells. If not using all at once, prepare aliquots and store at -20°C. Limit freeze-thaw cycles to three.

4. **HSA TSA Positive Control Assay Reagent - (Item No. 401201)**

   This vial contains 55 µl of a naproxen solution. The reagent is ready to use as supplied. One vial is sufficient for eight control wells or four separate experiments with duplicate control measurements. Aliquot as needed to limit freeze-thaw cycles to three and store remaining reagent at -20°C.

Sample Preparation

Aliquot and set aside 2 ml HSA TSA Buffer (2X) for use in preparation of the Master Mix (see Table 2 on page 12), and then dilute 10 ml of HSA TSA Buffer (2X) to a 1X solution (20 ml final) using pure water. Prepare the compound to be tested in the solvent of choice, then dilute in HSA TSA Buffer (1X) according to the table below to prepare a 4X analyte working stock. This ensures that the final solvent concentration in the assay remains within the established solvent tolerance and does not interfere with assay results.

Because binding to HSA can vary by compound, testing multiple concentrations of the test compound is recommended. Prepare additional concentrations by serially diluting the compound in the selected solvent before diluting in HSA TSA Buffer (1X) to prepare the 4X working stocks.

Solvent	Recommended Stock Concentration in 100% solvent*	Minimum Dilution Factor to Prepare 4X Analyte Working Stock
DMSO	50X	12.5X
Ethanol	20X	5X
Methanol	20X	5X

Table 1. Analyte working stock preparation

**Stock concentrations are relative to the desired final analyte concentration in the assay.*

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. It is suggested that each control, sample, and vehicle control be assayed at least in duplicate.

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 20 µl in all of the wells.
- The volume of prepared 4X analyte working stock needed per well is 5 µl.
- All reagents should be prepared as described in the **Reagent Preparation** section (see page 8) and kept at room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- The assay is performed over a temperature gradient *via* a qPCR instrument (keep samples at room temperature until measurement).
- Read the fluorescence with excitation and emission wavelengths of 468 and 507 nm, respectively, or using the SYBR® Green settings for the instrument.

Performing the Assay

1. Prepare the Master Mix as described in Table 2 below. Store at room temperature until use. The Master Mix should be protected from light and used within two hours of preparation. Scale accordingly.

Reagent	8 wells	24 wells	96 wells
HSA TSA Buffer (2X)	75 µl	225 µl	900 µl
TSA Probe	20 µl	60 µl	240 µl
TSA Human Serum Albumin	6 µl	18 µl	72 µl
Pure Water	49 µl	147 µl	588 µl
Final Volume	150 µl	450 µl	1,800 µl

Table 2. Master mix preparation

2. Pipette 15 µl of Master Mix to all wells.
3. Add the appropriate volume of prepared reagents to the designated wells according to Table 3 on page 13. Mix thoroughly, ensuring no bubbles are formed.

	Control Wells	Sample Wells	Vehicle Control Wells
HSA TSA Positive Control	5 µl	--	--
4X Analyte Working Stock	--	5 µl	--
Sample Vehicle	--	--	5 µl
Master Mix	15 µl	15 µl	15 µl

Table 3. Pipetting summary

4. Cap or seal the strip wells or plate and store at room temperature protected from light until running the assay (maximum one hour).
5. Program the qPCR instrument to perform a thermal shift assay using the SYBR® Green channel and set the passive reference to 'None'. Initiate the assay with a recommended hold at 37°C for 10 minutes, followed by a 0.015°C/second (or ~1°C/minute) temperature ramp to 95°C with measurements every 2 seconds.

ANALYSIS

Calculations

1. Determine the melting T_m values for vehicle controls and all tested concentrations of analytes.
   a. If using dedicated software for thermal shift assays, use the lowest T_m peak for evaluation of analyte:HSA interactions.
   b. For evaluation of the T_m values using the raw fluorescence *versus* temperature data, fit the data using a Boltzmann sigmoidal curve fit (see Figure 2 on page 15). The inflection point for the increase in fluorescence that occurs with the lowest temperature melting phase is the T_m sensitive to the thermal shift.
   c. For evaluation of the T_m values from the first derivative of the raw data (ΔFluorescence/ΔTemperature), the top of the lowest temperature peak represents the T_m sensitive to the thermal shift (see Figure 3 on page 16).

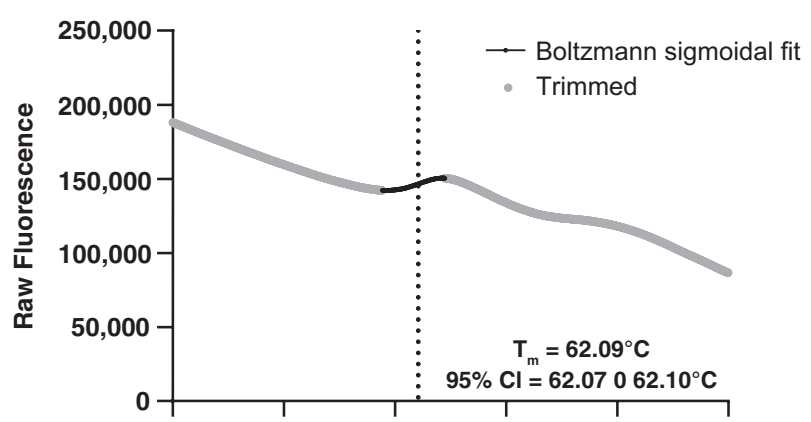


Figure 2. Raw fluorescence plot. Representation of the raw fluorescence data where a Boltzmann sigmoidal curve fit to the first inflection point is used to measure the T_m sensitive to analyte binding.

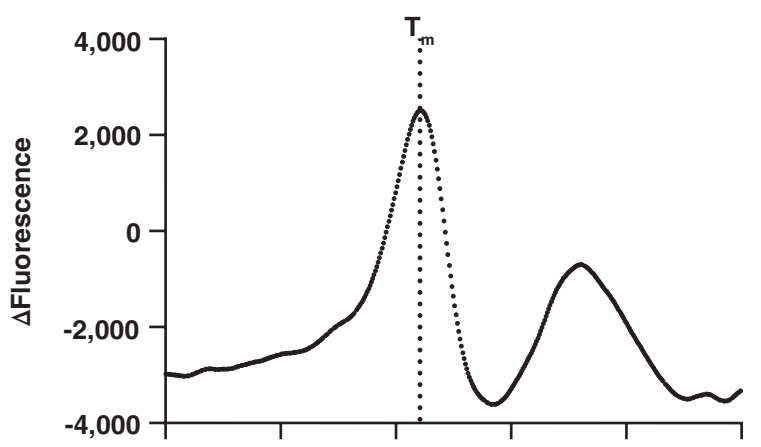


Figure 3. First derivative. The raw fluorescence data from Figure 2 transformed as the first derivative, where the inflection point is now the top of the lowest temperature peak.

2. Subtract the vehicle control T_m value from each sample to calculate the ΔT_m.
3. Plot the measured ΔT_m *versus* log [concentration]. A thermal shift is indicated by a concentration-dependent increase in ΔT_m relative to the vehicle control (see Figure 4 below). For analytes resulting in a thermal shift, perform linear regression on the linear portion of the thermal shift response and calculate the critical concentration for thermal shift from the x-intercept of the linear regression.
4. For comparison of multiple compounds, the critical concentration value can be represented similar to the example data shown in Figure 5 on page 18.

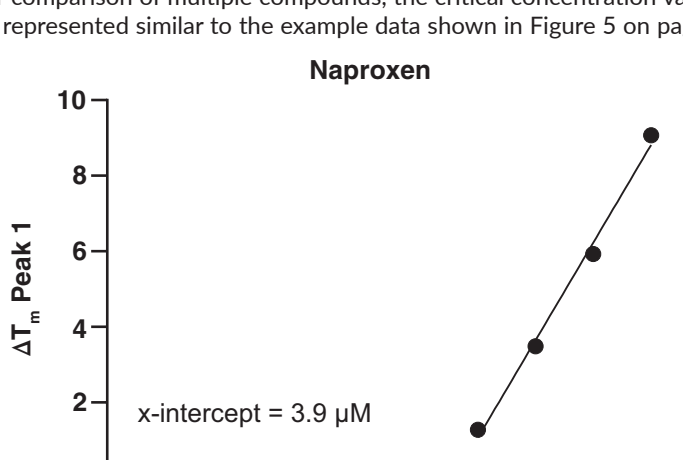


Figure 4. Peak 1 ΔT_m plot. A plot representing the ΔT_m relative to the vehicle control for naproxen for visualization of the thermal shift. The portion of the plot showing a linear increase in ΔT_m was fitted by linear regression and the x-intercept (critical concentration) value is indicated.

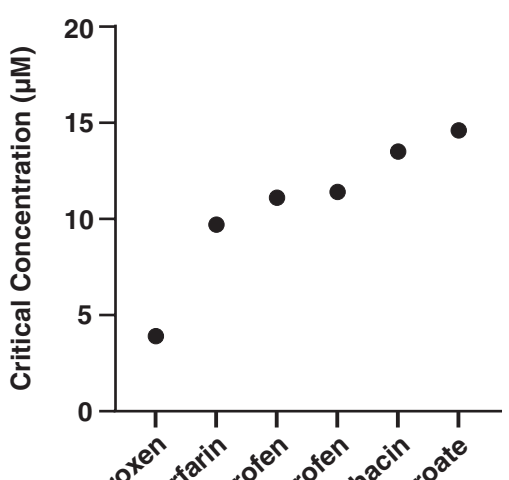


Figure 5. Thermal shift critical concentration plot. The critical concentration values, or the thermal shift x-intercepts from peak 1 ΔT_m plots, are represented together for a set of known HSA-binding compounds.

Performance Characteristics

Precision

When a series of four vehicle control measurements were performed on the same day, the intra-assay coefficient of variation was 0.15%. When a series of three vehicle control measurements were performed on different days under the same experimental conditions by different users, the inter-assay coefficient of variation was 0.21%.

Interferences

A reagent is considered to interfere if it results in >0.5°C difference in expected T_m.

Reagents		Will Interfere?
Solvents	DMSO (up to 2% final concentration)	No
	Ethanol (up to 5% final concentration)	No
	Methanol (up to 5% final concentration	No

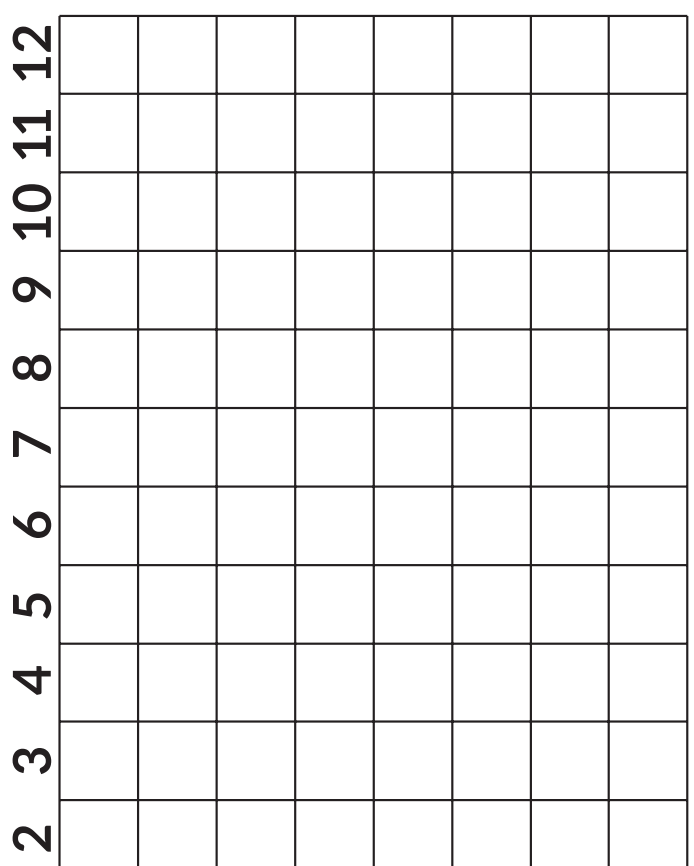
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of replicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate to remove bubbles
No thermal shift with analyte	A. Analyte does not bind to HSA B. Solvent concentration in sample wells does not match vehicle controls	A. Test higher concentrations of analyte if possible to detect weak binding B. Ensure solvent concentration matches across all samples and vehicle controls

References

1. Fanali, G., di Masi, A., Trezza, V., *et al.* Human serum albumin: From bench to bedside. *Mol. Aspects Med.* **33(3)**, 209-290 (2012).
2. Jackson, T.W., Scheibly, C.M., Polera, M.E., *et al.* Rapid characterization of human serum albumin binding for per- and polyfluoroalkyl substances using differential scanning fluorimetry. *Environ. Sci. Technol.* **55(18)**, 12291-12301 (2021).
3. Sabbioni, G. and Turesky, R.J. Biomonitoring human albumin adducts: The past, the present, and the future. *Chem. Res. Toxicol.* **30**, 332-366 (2017).
4. Tayyab, S. and Feroz, S.R. Serum albumin: Clinical significance of drug delivery vehicle. *Adv. Protein. Chem. Struct. Biol.* **123**, 193-218 (2021).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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