Cystine Uptake Assay Kit

Item No. 42921

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GENERAL INFORMATION

Materials Supplied

Item No.	Item	Quantity/Size	Storage
401058	Cystine Analog Solution	1 vial/225 µl	-20°C
401059	Cystine Uptake Fluorescent Probe	1 vial	-20°C
401061	Cystine Uptake Reducing Agent	2 vials	-20°C
401063	Cystine Uptake Assay Buffer	1 bottle/25 ml	-20°C
400012	96-Well Cover Sheet	1 cover	RT

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.

Please tap the tubes before opening and open with care. The contents may have relocated from the bottom of the tube during shipment.

This kit may not perform as described if any reagent or procedure is replaced or modified.

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 plate reader capable of measuring fluorescence with excitation and emission wavelengths of 490 and 535 nm, respectively.
2. 96-well, black, clear bottom cell culture plate
3. Materials needed for cell culture, including cystine- and serum-free medium and PBS.
4. Methanol
5. DMSO
6. A source of pure water; glass-distilled water or pure water is acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*

INTRODUCTION

Background

Cystine is the oxidized, dimeric form of cysteine and an essential component of the extracellular cystine/cysteine redox couple.[1] In its anionic form, it is imported into cells primarily by the system x_c^- cystine/glutamate transporter (xCT) and rapidly reduced by thioredoxin reductase 1 (TrxR1) to cysteine, which is required for glutathione biosynthesis.[1,2] Plasma cystine levels increase with age, and high plasma levels of cystine are associated with the risk of major cardiovascular events, including heart attack and death.[3] Cystine accumulates in the lysosomes of patients with cystinosis, an autosomal recessive lysosomal storage disorder associated with renal Fanconi syndrome and loss of glomerular function.[4] Inhibition of cystine import leads to a reduction in glutathione levels, an increase in oxidative stress, and, ultimately, ferroptotic cell death.[1]

About This Assay

The Cystine Uptake Assay Kit is a convenient fluorescence-based assay for studying cellular uptake of cystine *via* xCT without the use of radioisotopes. The cystine analog (CA) in this kit is taken up into cells *via* xCT, and the transported CA is specifically detected using the provided Fluorescent Probe and Reducing Agent (See Figure 1, below).[5]

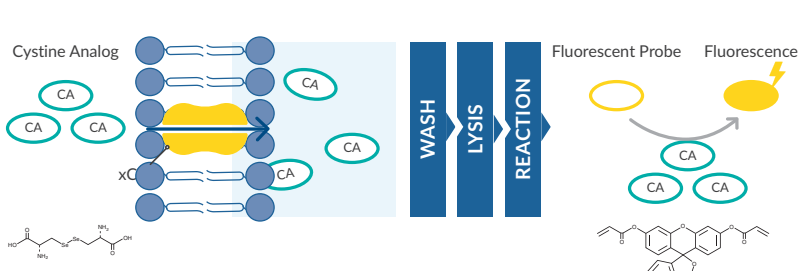

Figure 1. Principle of the Cystine Uptake Assay Kit

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Cystine Analog Solution - (Item No. 401058)**

   Dilute the Cystine Analog 100-fold by adding 200 µl to 20 ml of cystine- and serum- free medium. This is a sufficient volume to assay an entire plate. Scale as needed. Warm to 37°C prior to use as uptake may be affected by the temperature of this solution. Use the CA uptake solution within 6 hours.

2. **Cystine Uptake Fluorescent Probe - (Item No. 401059)**

   Centrifuge the tube briefly before opening to remove all contents from the walls and cap. Reconstitute with 50 µl of DMSO and vortex. Store the fluorescent probe solution protected from light at -20°C, where it will be stable for two months.

3. **Cystine Uptake Reducing Agent - (Item No. 401061)**

   Centrifuge the tube briefly before opening to remove all contents from the walls and cap. Reconstitute each vial with 100 µl of pure water and vortex. Store the reducing agent solution protected from light at -20°C, where it will be stable for two months.

4. **Cystine Uptake Assay Buffer - (Item No. 401063)**

   This vial contains 25 ml of Cystine Uptake Assay Buffer. It is ready to use as supplied.

GENERAL PROTOCOL

Adherent Cells Protocol

Use a multichannel pipette to reduce the time difference between loading each well. Warm the culture medium and CA uptake solution to 37°C prior to use as cystine uptake into cells may be affected by temperature. It is recommended that each sample/treatment and the corresponding background be run in triplicate.

1. Seed 10^4-10^5 cells in a 96-well black, clear bottom cell culture microplate and allow the cells to adhere at 37°C overnight in a 5% CO_2 incubator.
2. Remove the culture medium and gently wash the cells three times with warm cystine- and serum-free medium.
3. Pre-treat the cells by adding the reagents to the designated wells according to Table 1 below. Administer the treatment and the corresponding vehicle control as specified in the experimental design. Cover the plate and incubate at 37°C for 5 minutes in a 5% CO_2 incubator.

	Sample Background Wells	Vehicle Control Wells	Sample Wells
Warm cystine- and serum-free media	200 µl	198 µl	198 µl
Vehicle control	--	2 µl	--
Treatment	--	--	2 µl

Table 1. Pre-treatment pipetting summary

4. Remove the supernatant and add the reagents to the designated wells according to Table 2 below. Administer the treatment and the corresponding vehicle control as specified in the experimental design.

	Sample Background Wells	Vehicle Control Wells	Sample Wells
Warm cystine- and serum-free media	200 µl	--	--
Warm CA uptake solution	--	198 µl	198 µl
Vehicle control	--	2 µl	--
Treatment	--	--	2 µl

Table 2. CA uptake pipetting summary

5. Cover the plate and incubate at 37°C for 30 minutes in a 5% CO_2 incubator.
6. Remove the supernatant and wash the cells with 200 µl of ice-cold PBS three times.
7. Remove the supernatant and add 50 µl of methanol.
8. Prepare the working solution immediately before use according to Table 3, on page 11.

	100 tests
Reconstituted Fluorescent Probe	40 µl
Reconstituted Reducing Agent	400 µl
Assay Buffer	20 ml

Table 3. Working solution preparation

9. Add 200 µl of working solution, mix by pipetting, seal the plate, and incubate at 37°C for 30 minutes.
10. Measure the fluorescence intensity using a fluorescence plate reader (Ex/Em = 490/535 nm).
11. Subtract the measured value of the sample background from the value measured for each sample.

Optional: If you cannot obtain a difference in fluorescence intensity between samples or with sample background wells, correct it using a nuclear staining method, such as DAPI or Hoechst.

Suspension Cells Protocol

Use a multichannel pipette to reduce the time difference between loading each well. Let the culture medium and CA uptake solution warm up to 37°C prior to use as cystine uptake into cells may be affected by temperature. It is recommended that each sample/treatment and the corresponding background be run in triplicate

1. Dispense 10^4-10^5 cells into 96-well v-bottom microplate.
2. Centrifuge the cells at 300 × *g* for 3 minutes.
3. After removing the supernatant, add 200 µl of warm medium (cystine- and serum-free; 37°C), pipet to resuspend and wash the cell pellet, and centrifuge at 300 x *g* for 3 minutes. Repeat this step two more times.
4. Remove the supernatant and pre-treat the cells by adding the reagents to the designated wells according to Table 4 below. Administer the treatment and the corresponding vehicle control as specified in the experimental design. Incubate at 37°C for 5 minutes in a 5% CO_2 incubator.

	Sample Background Wells	Vehicle Control Wells	Sample Wells
Warm cystine- and serum-free media	200 µl	198 µl	198 µl
Vehicle control	--	2 µl	--
Treatment	--	--	2 µl

Table 4. Pre-treatment pipetting summary

5. Centrifuge the cells at 300 x *g* for 3 minutes and remove the supernatant. Add the reagents to the designated wells according to Table 5 below. Administer the treatment and the corresponding vehicle control as specified in the experimental design.

	Sample Background Wells	Vehicle Control Wells	Sample Wells
Warm cystine- and serum-free media	200 µl	--	--
Warm CA uptake solution	--	198 µl	198 µl
Vehicle control	--	2 µl	--
Treatment	--	--	2 µl

Table 5. CA uptake pipetting summary

6. Resuspend the cells and incubate at 37°C for 30 minutes in a 5% CO_2 incubator.
7. Centrifuge at 300 x *g* for 3 minutes and remove the supernatant. Wash the cells with 200 µl ice-cold PBS. Repeat this step two more times.
8. After removing the supernatant, add 100 µl of methanol and mix by pipetting. *NOTE: To correct the measured value for protein concentration, use 50 µl of the solution from this step for the cystine uptake measurement and use the remaining portion for the protein concentration measurement.*

9. Prepare the working solution immediately before use. See Table 6, below.

	100 tests
Reconstituted Fluorescent Probe	40 µl
Reconstituted Reducing Agent	400 µl
Assay Buffer	20 ml

Table 6. Working solution preparation

10. Transfer 50 µl of the solution from step 8 to a 96-well black microplate and add 200 µl of the working solution. Mix by pipetting. Seal the plate and incubate at 37°C for 30 minutes.
11. Measure the fluorescence intensity using a fluorescence plate reader (Ex/Em = 490/535 nm).
12. Subtract the measured value of the sample background from the value measured for each sample.

EXPERIMENTAL EXAMPLES

Adherent Cells

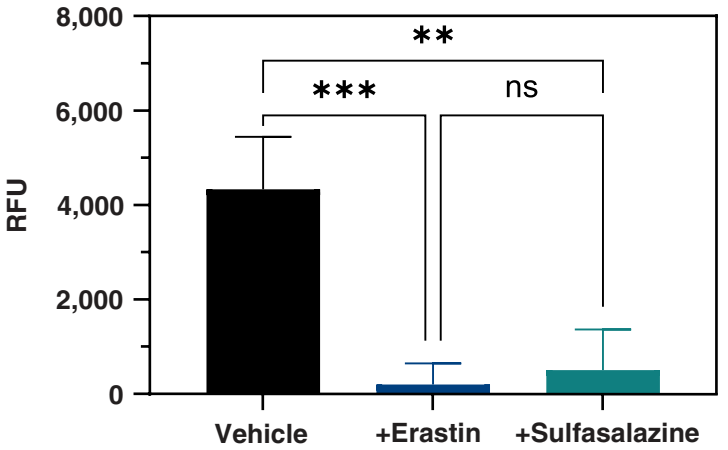

Figure 2. Effect of xCT inhibitors erastin and sulfasalazine on cystine uptake in HeLa cells. HeLa cells (1 x 10^5 cells/well) were treated with DMSO (Vehicle), 100 µM erastin, or 100 µM sulfasalazine (Item Nos. 17754 and 15025, respectively) and processed as described in the **Adherent Cells Protocol** (on page 9). The fluorescence intensity was measured using a fluorescence plate reader with excitation/emission wavelengths of 490 and 535 nm, respectively.

Suspension Cells

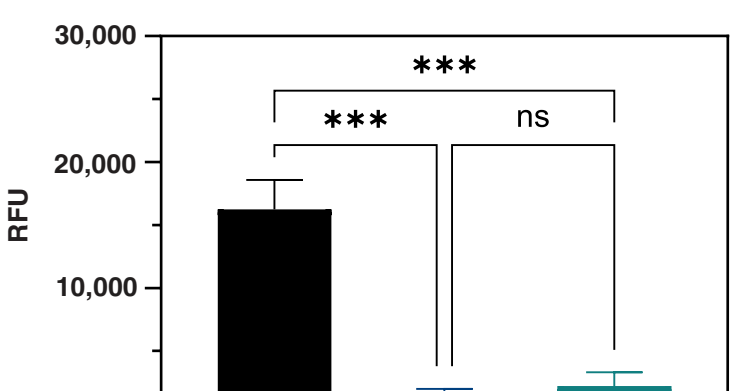

Figure 3. Effect of xCT inhibitors erastin and sulfasalazine on cystine uptake. HL60 cells (4 x 10^5 cells/well) were treated with DMSO (Vehicle), 100 µM erastin, or 100 µM sulfasalazine and processed as described in the **Suspension Cells Protocol** (on page 12). The fluorescence intensity was measured using a fluorescence plate reader with excitation/emission wavelengths of 490 and 535 nm, respectively.

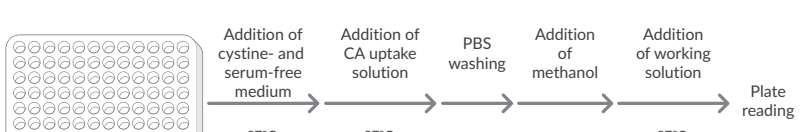

Figure 4. Protocol summary

References

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2. Conrad, M. and Sato, H. The oxidative stress-inducible cystine/glutamate antiporter, system x_c^-: Cystine supplier and beyond. *Amino Acids* **42**, 231-246 (2021).
3. Go, Y.-M. and Jones, D.P. Cysteine/cystine redox signaling in cardiovascular disease. *Free Radic. Biol. Med.* **50(4)**, 495-509 (2011).
4. Elmonem, M.A., Veys, K.R., Soliman, N.A., *et al.* Cystinosis: A review. *Orphanet J. Rare Dis.* **11**, 47 (2016).
5. Shimomura, T., Hirakawa, N., and Ohuchi, Y., *et al.* Simple fluorescence assay for systine uptake via the xCT in cells using selenocystine and a fluorescent probe. *ACS Sens.* **6**, 2125-2128 (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.