

**Submission ID #: 70104**

**Scriptwriter Name: Deepthi Das**

**Project Page Link: [https://review.jove.com/files\\_upload.php?src=21381768](https://review.jove.com/files_upload.php?src=21381768)**

**Title: Colorimetric Assessment of Deiodinase 1 Activity in Human Liver Microsomes Using the Sandell-Kolthoff Reaction**

**Authors and Affiliations:**

**Konstantinos Stefanidis<sup>1,2</sup>, Nina Hambruch<sup>1</sup>, Kostja Renko<sup>3</sup>, Dorothee Funk-Weyer<sup>1</sup>, Robert Landsiedel<sup>1,2</sup>**

<sup>1</sup>Experimental Toxicology and Ecology, BASF SE

<sup>2</sup>Pharmacy, Pharmacology and Toxicology, Free University of Berlin

<sup>3</sup>German Federal Institute for Risk Assessment (BfR), German Centre for the Protection of Laboratory Animals (Bf3R)

**Corresponding Authors:**

Nina Hambruch [nina.hambruch@basf.com](mailto:nina.hambruch@basf.com)

**Email Addresses for All Authors:**

Konstantinos Stefanidis [konstantinos.stefanidis@basf.com](mailto:konstantinos.stefanidis@basf.com)

Kostja Renko [kostja.renko@bfr.bund.de](mailto:kostja.renko@bfr.bund.de)

Dorothee Funk-Weyer [dorothee.funk-weyer@basf.com](mailto:dorothee.funk-weyer@basf.com)

Robert Landsiedel [robert.landsiedel@basf.com](mailto:robert.landsiedel@basf.com)

Nina Hambruch [nina.hambruch@basf.com](mailto:nina.hambruch@basf.com)

## Author Questionnaire

1. **Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**
3. **Filming location:** Will the filming need to take place in multiple locations? **No**
4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live** during your JoVE shoot? **No**

### Current Protocol Length

Number of Steps: 16

Number of Shots: 47

# Introduction

---

*Videographer: Obtain headshots for all authors available at the filming location.*

## INTRODUCTION:

**REQUIRED:** Summarize your research focus and the main questions it aims to answer.

- 1.1. **Konstantinos Stefanidis:** The assay quantifies DIO1 inhibition using human liver microsomes and was validated and optimized for robust and standardized handling.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Konstantinos Stefanidis:** This protocol is used in mechanistic toxicology and screening to identify chemicals that inhibit DIO1 and disrupt thyroid hormone homeostasis.

## CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Konstantinos Stefanidis:** This protocol measures iodide release from rT3 through deiodination via the Sandell–Kolthoff reaction, a practical surrogate readout of DIO1 activity.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Konstantinos Stefanidis:** Maintaining strict, uniform handling is a key challenge, as slight variations in timing and handling can significantly affect protocol efficiency.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Konstantinos Stefanidis:** Future studies can adapt this standardized workflow to other sources of deiodinating enzymes, enabling assays for DIO2, DIO3 or dehalogenases.

*Videographer: Obtain headshots for all authors available at the filming location.*

# Protocol

---

## 2. Pre-Assay Work: Casting of Ion Exchange Resin

**Demonstrator:** Konstantinos Stefanidis

2.1. To begin, obtain an appropriate rectangular transparent container [1]. Add about 250 grams of ion exchange resin to the container [2]. Wash the resin with 10 percent acetic acid and let the resin suspension rest for 10 minutes [3]. Then, slightly tilt the container to aspirate the supernatant [4]. Repeat the wash at least 5 times in total, or until no more color leaks into the solvent [5].

2.1.1. WIDE: Show the experimental set up and then show the talent placing the rectangular container on the work surface.

2.1.2. Talent adding ion exchange resin to the container.

2.1.3. Talent washing the resin with 10% acetic acid and leaving the resin suspension to rest.

2.1.4. Talent slightly tilting the container and aspirating the supernatant.

2.1.5. Show the talent restarting the washing procedure and then show the washed resin with the clear solvent. Author's/videographer's NOTE: 2.1.5 was broken into 2 takes. Take 1 is talent doing the washing procedure and Take 2 is close up of the resin container since there were essentially 2 different steps.

2.2. After the last wash, add 10 percent acetic acid so it makes up approximately 50 percent of the total volume [1]. Tilt the container so the resin moves to one side [2]. Stabilize the container in the tilted position and wait about 10 minutes for the phases to separate [3].

2.2.1. Talent adding an appropriate volume of 10% acetic acid to the washed resin.

2.2.2. Talent tilting the container so the resin collects on one side.

2.2.3. Talent stabilizing the tilted container for phase separation. Author's/videographer's NOTE: 2.2.2 and 2.2.3 are combined and slated as 2.2.2.

2.3. Place a 96-well filter plate on top of a 96-deep well plate [1]. Add 100 microliters of 10 percent acetic acid into each well of the 96-well filter plate [2].

2.3.1. Talent placing a 96-well filter plate on top of a 96-deep well plate.

2.3.2. Talent adding 100 microliters of 10% acetic acid into the wells of the 96-well filter plate.

- 2.4. Cut the tips used for resin casting at a height of about 1 centimeter to widen the opening at the end [1]. Use the cut tip to cast 300 microliters of ion exchange resin into each well of the 96-well filter plate [2]. Use a centrifuge with a swing-out rotor for microtiter plates to elute acetic acid from the filter plate into a 96-deep well plate at 100 to 200 relative centrifugal force for 1 minute [3]. Check whether the resin is distributed evenly across the plate [4]. **NOTE: Text Overlay has been merged with VO.**

- 2.4.1. Talent cutting the casting tips to widen the opening.
- 2.4.2. Talent dispensing ion exchange resin into the wells of the 96-well filter plate.
- 2.4.3. Talent placing the plate assembly into the centrifuge.  
~~**TXT: Centrifuge at 100–200 g, 1 min**~~
- 2.4.4. Talent inspecting the resin distribution across the plate.

- 2.5. Seal the plate with an impermeable sheet of plastic [1]. Store the plate at 4 degrees Celsius for a maximum of 6 months [2]. **NOTE: Text Overlay has been merged with VO.**

- 2.5.1. Talent sealing the plate with an impermeable sheet of plastic.
- 2.5.2. Talent storing the sealed plate at 4°C. ~~**TXT: Maximum storage: 6 months**~~

### 3. Pre-Assay Work: Standardization of Sandell-Kolthoff reaction

- 3.1. Prepare iodide dilutions from the iodide source in deionized water as presented [1]. Add 50 microliters of pure deionized water as a control to a 96-well plate in triplicate [2]. Add 50 microliters of the prepared iodide dilutions to the same plate in triplicate [3].

- 3.1.1. TEXT IN PLAIN BACKGROUND  
Iodide dilutions (nM):  
1500, 1000, 750, 500, 400, 300, 200, 100, 50, 25, 10, 5, 1
- 3.1.2. Talent dispensing pure deionized water into control wells of a 96-well plate.
- 3.1.3. Talent dispensing the iodide dilutions into the remaining wells in triplicate.  
*Video Editor: Please show Figure 1 as an inset.*

- 3.2. Now, add 50 microliters of 40 millimolar cerium solution to all wells [1]. Then, start the reaction by adding 50 microliters of 25 millimolar arsenite solution to all wells [2]. Immediately after this, measure the absorbance with a plate reader at 415 nanometers [3]. Record the initial optical density and then record the optical density every minute for 21 minutes [4].

- 3.2.1. Talent adding 40 mM cerium solution to all wells.

- 3.2.2. CU: Talent adding 25 mM arsenite solution to all wells.
- 3.2.3. Talent placing the plate into the plate reader for measurement at 415 nm.
- 3.2.4. Shot of the plate reader recording the initial optical density and show a timer being set for 21 min. *Videographer: Please record the screen of the plate reader.*

#### **4. Performing the Deiodinase1-Sandell-Kolthoff (DIO1-SK) assay**

- 4.1. Prepare the reference item, the positive control, the negative control, and the test items at suitable concentrations as described [1].

- 4.1.1. TEXT ON PLAIN BACKGROUND:

**Reference item: 6-Propyl-2-thiouracil**

10<sup>-1</sup> molar stock, serial dilutions

**Positive control: Aurothioglucose**

10<sup>-2</sup> molar stock, 10<sup>-3</sup> molar working

**Negative control: 1-Thio-β-D-glucose sodium salt**

10<sup>-2</sup> molar stock, 10<sup>-3</sup> molar working

**Test items: Chemicals under evaluation**

Serial dilutions

- 4.2. Prepare the substrate mix and a microsome suspension [1]. Keep the microsome suspension on ice until incubation [2].

- 4.2.1. Shot of the freshly prepared substrate mix and a microsome suspension with the labels clearly visible.

- 4.2.2. Talent placing the microsome suspension on ice. **Author's/videographer's NOTE: 4.2.1 and 4.2.2 are combined and slated as 4.2.1.**

- 4.3. Add 10 microliters of all the reference item concentrations, various controls and the test item concentrations to the wells according to the plate layout [1]. Add 40 microliters of the microsome suspension to all wells [1]. Add 50 microliters of the freshly prepared substrate mix to the samples [2].

- 4.3.1. Representative shot of talent dispensing 10 μL of each solution into the wells according to the plate layout. *Video Editor: Please show Figure 3 as an inset.*

- 4.3.2. Shot of talent adding the microsome suspension to the wells.

- 4.3.3. Talent adding the freshly prepared substrate mix to the samples.

- 4.4. Seal the plate with an impermeable sheet of plastic [1]. Place it on a shaker in an incubator at 37 degrees Celsius and 850 revolutions per minute for 2 hours [2]. Then

place the plate on ice to stop the reaction and leave it there until measurement [3].

NOTE: Text Overlay has been merged with VO.

4.4.1. Talent sealing the plate with an impermeable sheet of plastic.

4.4.2. Talent placing the plate in the incubator shaker ~~TXT: 37 °C, 850 rpm, 2 h~~

Author's/videographer's NOTE: 4.4.1 and 4.4.2 are combined in one shot.

4.4.3. Talent taking the plate from the incubator and keeping it on ice.

## 5. Separation Of Iodine Using Ion Exchange Resin-Filled Filter Plates

5.1. Put the ion exchange resin-filled 96-well filter plate on top of a 96-deep well plate [1]. Add 150 microliters of 10 percent acetic acid to each well of the filter plate to wet the resin in the columns [2]. Use a centrifuge with a swing-out rotor for microtiter plates to elute acetic acid from the filter plate into a 96-deep well plate at 100 to 200 relative centrifugal force for 1 minute [3]. Replace the used 96-deep well plate with a new 96-deep well plate [4]. NOTE: Text Overlay has been merged with VO.

5.1.1. Talent placing the ion exchange resin-filled 96-well filter plate on top of a 96-deep well plate.

5.1.2. Talent adding 150  $\mu$ L of 10% acetic acid to each well resin-filled 96-well filter plate.

5.1.3. Talent placing the plate assembly into the centrifuge. ~~TXT: Centrifuge at 100–200 g, 1 min~~

5.1.4. Talent replacing the used 96-deep well plate with a new 96-deep well plate.

5.2. Now, collect the plate previously left on ice for incubation [1]. Add 133 microliters of 10 percent acetic acid to each well of this 96-well plate [2]. Shake the plate briefly to mix the solutions [3]. Transfer 175 microliters of these samples to the ion exchange resin-filled 96-well filter plate, maintaining the original plate layout [4].

5.2.1. Talent collecting the plate previously left on ice.

5.2.2. Talent adding 133  $\mu$ L of 10% acetic acid to each well of this 96-well plate.

5.2.3. Talent briefly shaking the plate to mix the solutions.

5.2.4. Talent transferring the prepared samples onto the ion exchange resin-filled 96-well filter plate. *Video Editor: Please show Figure 3 as an inset.*

5.3. Use a centrifuge, with a swing-out rotor for microtiter plates, to pass the samples through the resin into the 96-deep well plate at 100 to 200 relative centrifugal force for 1 minute [1]. Remove the ion exchange resin-filled 96-well filter plate [2]. NOTE: Text Overlay has been merged with VO.

- 5.3.1. Talent placing the plate assembly into the centrifuge. ~~TXT: Centrifuge at 100–200 g, 1 min~~
- 5.3.2. Talent removing the ion exchange resin-filled 96-well filter plate.

## **6. Sandell–Kolthoff reaction for iodide quantification**

- 6.1. From the 96-deep well plate previously prepared, transfer 50 microliters of the undiluted sample in an unused 96-well plate [1]. Add 50 microliters of 40 millimolar cerium solution to all wells [2]. Start the reaction by adding 50 microliters of 25 millimolar arsenite solution to all wells [3].
  - 6.1.1. Talent transferring 50µL of the undiluted samples to a novel 96-well plate.
  - 6.1.2. Talent adding 40 mM cerium solution to all wells.
  - 6.1.3. CU: Talent adding 25 mM arsenite solution to all wells.
- 6.2. Immediately after this, measure the absorbance with a plate reader at 415 nanometers [1]. Record the initial optical density and then record the optical density every minute for 21 minutes [2].
  - 6.2.1. Talent placing the plate into the plate reader for measurement at 415 nm.
  - 6.2.2. Shot of the plate reader recording the initial optical density and the optical density every minute for 21 minutes. *Videographer: Please record the screen of the plate reader.*

# Results

---

## 7. Results

7.1. The background-corrected Sandell-Kolthoff signal increased with increasing microsomal protein [1]. The signal varied across post-ion-exchange dilutions [2]. The 1:2 dilution yielded the highest signal while maintaining a sigmoidal response [3].

7.1.1. LAB MEDIA: Figure 7A. *Video editor: Please emphasize the increasing trend on all three lines*

7.1.2. LAB MEDIA: Figure 7A. *Video editor: Please highlight the 3 dilution labels in the legend: undiluted, 1:2 dilution, and 1:4 dilution*

7.1.3. LAB MEDIA: Figure 7A. *Video editor: Please highlight the curve and legend entry for the 1:2 dilution*

7.2. Comparison of inhibition curves identified 5 micrograms of microsomal protein per well as the lowest amount producing a sigmoidal response [1].

7.2.1. LAB MEDIA: Figure 7B. *Video editor: Please highlight the yellow curve and legend entry for 5 microgram protein per well*

7.3. The reference inhibitor showed a concentration-dependent decrease in iodide release activity with a sigmoidal inhibition profile [1], tannic acid showed a comparable inhibitory profile with high maximum inhibition [2], and dibutyl phthalate showed no inhibition across the tested concentration range [3]. These results showed that the assay clearly distinguished inhibitory from non-inhibitory concentration–response profiles under standardized conditions [4].

7.3.1. LAB MEDIA: Figure 8A. *Video editor: Please highlight the downward sloping curve in panel A.*

7.3.2. LAB MEDIA: Figure 8B. *Video editor: Please highlight the curve in panel B*

7.3.3. LAB MEDIA: Figure 8C. *Video editor: Please highlight the flat line in panel C for dibutyl phthalate*

7.3.4. LAB MEDIA: Figure 8. *Video editor: Show all three panels A, B, and C side by side to contrast the different curve shapes*

## Pronunciation guides:

1. Ion exchange resin

Pronunciation link: <https://www.merriam-webster.com/dictionary/resin>

IPA: /'rezɪn/

Phonetic Spelling: reh-zuhn

2. Acetic

Pronunciation link: <https://www.merriam-webster.com/dictionary/acetic>

IPA: /ə'si:tɪk/

Phonetic Spelling: uh-see-tihk

3. Aspirate

Pronunciation link: <https://www.merriam-webster.com/dictionary/aspirate>

IPA: /'æspə'reɪt/

Phonetic Spelling: as-puh-rayt

4. Supernatant

Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>

IPA: /,su:pər'neɪtənt/

Phonetic Spelling: soo-per-nay-tuhnt

5. Microliters

Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>

IPA: /'maɪkroʊ'li:tər/

Phonetic Spelling: my-kroh-lee-ter

6. Centrifuge

Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>

IPA: /'sentrə'fju:dʒ/

Phonetic Spelling: sen-truh-fyooj

7. Rotor

Pronunciation link: <https://www.merriam-webster.com/dictionary/rotor>

IPA: /'roʊtər/

Phonetic Spelling: roh-ter

8. Elute

Pronunciation link: <https://www.merriam-webster.com/dictionary/elute>

IPA: /i'lu:t/

Phonetic Spelling: ee-loot

9. Impermeable

Pronunciation link: <https://www.merriam-webster.com/dictionary/impermeable>

IPA: /ɪm'pɜ:miəbl/

Phonetic Spelling: im-per-mee-uh-buhl

10. Sandell-Kolthoff

Pronunciation link: No confirmed link found

IPA: /sæn'del 'kɒlt,hɔ:f/

Phonetic Spelling: san-del kohlt-hoff

11. Standardization

Pronunciation link: <https://www.merriam-webster.com/dictionary/standardization>

IPA: /,stændərdaɪ'zeɪʃən/

Phonetic Spelling: stan-der-dy-zay-shuhn

12. Iodide  
Pronunciation link: <https://www.merriam-webster.com/dictionary/iodide>  
IPA: /'aɪəˌdaɪd/  
Phonetic Spelling: eye·uh·dyde
13. Deionized  
Pronunciation link: <https://www.merriam-webster.com/dictionary/deionized>  
IPA: /di'aɪəˌnaɪzd/  
Phonetic Spelling: dee·eye·uh·nyzd
14. Cerium  
Pronunciation link: <https://www.merriam-webster.com/dictionary/cerium>  
IPA: /'sɪriəm/  
Phonetic Spelling: seer·ee·uhm
15. Arsenite  
Pronunciation link: <https://www.merriam-webster.com/dictionary/arsenite>  
IPA: /'ɑːrsəˌnaɪt/  
Phonetic Spelling: ar·suh·nyte
16. Absorbance  
Pronunciation link: <https://www.merriam-webster.com/dictionary/absorbance>  
IPA: /əb'zɔːrbəns/  
Phonetic Spelling: ub·zor·buhns
17. Nanometers  
Pronunciation link: <https://www.merriam-webster.com/dictionary/nanometer>  
IPA: /'nænəˌmi:tər/  
Phonetic Spelling: nan·uh·mee·ter
18. Optical density  
Pronunciation link: <https://www.merriam-webster.com/dictionary/optical>  
IPA: /'ɑːptɪkəl/  
Phonetic Spelling: op·tih·kuhl
19. Deiodinase  
Pronunciation link: No confirmed link found  
IPA: /diˌaɪ'ɒdəˌneɪs/  
Phonetic Spelling: dee·eye·oh·duh·nays
20. Microsome  
Pronunciation link: <https://www.merriam-webster.com/dictionary/microsome>  
IPA: /'maɪkrəˌsoʊm/  
Phonetic Spelling: my·kruh·sohm
21. Incubation  
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubation>  
IPA: /ˌɪŋkjə'beɪʃən/  
Phonetic Spelling: ing·kyuh·bay·shuhn
22. Sigmoidal  
Pronunciation link: <https://www.merriam-webster.com/dictionary/sigmoidal>

IPA: /sɪg'mɔɪdəl/

Phonetic Spelling: sig·moy·duhl

23. Microsomal

Pronunciation link: <https://www.merriam-webster.com/dictionary/microsomal>

IPA: /ˌmaɪkrə'soʊməl/

Phonetic Spelling: my·kruh·soh·muhl

24. Inhibitory

Pronunciation link: <https://www.merriam-webster.com/dictionary/inhibitory>

IPA: /ɪn'hɪbəˌtɔːri/

Phonetic Spelling: in·hib·uh·tor·ee

25. Dibutyl phthalate

Pronunciation link: <https://www.merriam-webster.com/dictionary/phthalate>

IPA: /'θæleɪt/

Phonetic Spelling: thal·ayt