DetectX® Superoxide Dismutase (SOD)

Colorimetric Activity Kit

2 Plate Kit – Catalog No. K028-H1

Species Independent

Sample Types Tested:
Serum, Plasma, Cells, Tissues,
and Erythrocyte Lysates

Please read this insert completely prior to using the product. For research use only.
Not for use in diagnostic procedures.
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TABLE OF CONTENTS

SUPPLIED COMPONENTS & STORAGE

		K028-H1	Description
Clear Half Area 96-well Plate	Quantity	2	Non-treated half-area 96-well plates
	Catalog No.	X018-2EA	
Superoxide Dismutase Standard*	Quantity	50 µL	SOD at 9,200 U/mL in stabilizing solution
	Catalog No.	C098-50UL	
Assay Buffer	Volume	50 mL	Buffer containing detergents, stabilizers, and dye.
	Catalog No.	X100-50ML	
Xanthine Oxidase Buffer	Volume	6 mL	Buffer containing detergents and stabilizers.
	Catalog No.	X102-6ML	
Xanthine Oxidase Concentrate 25X	Volume	225 µL	25X concentrated suspension of Xanthine Oxidase that must be diluted
	Catalog No.	C099-225UL	
Substrate Diluent	Volume	12 mL	Substrate buffer. **Keep tightly capped.**
	Catalog No.	X101-12ML	
Substrate Concentrate 10X	Volume	1.1 mL	10X concentrate that must be diluted
	Catalog No.	C100-1.1ML	

The unopened kit must be stored at -20˚C. Once opened, the kit can be stored at 4˚C up to the expiration date on the kit label, except for the SOD Standard, which must be stored at -20˚C.

*Aliquot the standard in high-quality polypropylene tubes prior to avoid multiple freeze-thaw cycles.

OTHER MATERIALS REQUIRED

- Distilled or deionized water
- Adjustable pipettes with disposable tips capable of dispensing 10 µL, 25 µL, 50 µL, 100 µL, and 1000 µL. Repeater pipettes or multichannel pipettes with corresponding tips are also recommended.
- Amber tubes sufficient to store 1X Xanthine Oxidase and 1X Substrate working solutions
- Glass or high-quality polypropylene test tubes for standard and sample preparation
- A plate reader capable of reading optical density at 450 nm
- Software for converting optical density (OD) readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.
- Optional: 1X Phosphate Buffered Saline (1X PBS; for cell and tissue samples)
- Optional: Potassium Cyanide solution (inhibition of Cu/Zn and extracellular SOD)
- Optional: Protease inhibitors such as phenylmethylsulfonyl fluoride (PMSF; for cell samples).

PRECAUTIONS

- Read this insert completely prior to using the product.
- This kit may not perform as described if any reagent or procedure is replaced or modified. Do not interchange reagents from different kit lots.
- Take appropriate safety precautions, such as: avoid breathing fumes, wear personal protective equipment (gloves, clothing, eye, and face protection), and familiarize yourself with SDS documents: https://www.arborassays.com/documentation/msds/K028-H1_MSDS.pdf

BACKGROUND

Short-lived and highly reactive oxygen species (ROS) such as $O2^{\cdot-}$ (superoxide), $^{\cdot}OH$ (hydroxyl radical), and H_2O_2 (hydrogen peroxide) are continuously generated *in vivo*. In the resting state, the balance between antioxidants and oxidants is sufficient to prevent the disruption of normal physiologic functions. However, changes in the levels of these two can disrupt this balance, resulting in abnormal levels of ROS and causing oxidative stress[1,2]. Cellular levels of ROS are controlled by antioxidant enzymes and small molecule antioxidants, with one of the major enzymes being Superoxide Dismutase (SOD).

SOD catalyzes the reduction of superoxide anions to hydrogen peroxide and oxygen. There are three types of SOD isoenzymes which are classified based on their metal cofactor: Fe-SOD (localized to chloroplasts), Mn-SOD (localized to mitochondria), and Cu/Zn-SOD (localized to chloroplasts, peroxisomes, and cytosol).[3] A fourth type, extracellular SOD (EC-SOD), contains a copper and zinc atom per subunit. It is a secreted protein and localized primarily within the extracellular matrix and at cell surfaces[4]. All four types of SOD play a critical role in scavenging superoxides and minimizing oxidative stress. Abnormal SOD activity is observed in various diseases. Increased SOD activity levels are seen in Down Syndrome[5.] Decreased SOD activity leads to harmful levels of oxidative stress and contributes to diseases such as diabetes, Alzheimer's disease, rheumatoid arthritis, Parkinson's disease, uremic anemia, atherosclerosis, some cancers, and thyroid dysfunction[6-11].

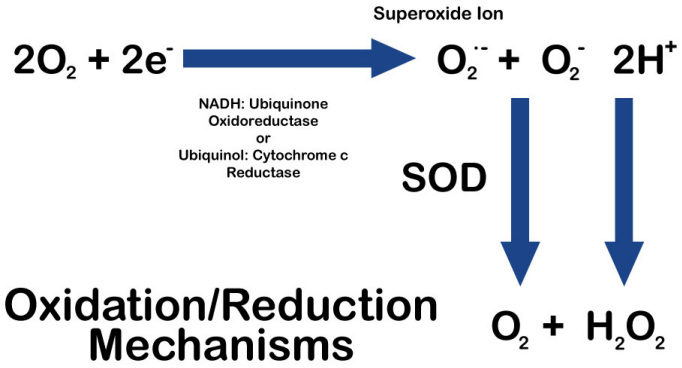


Figure 1: Reduction of superoxide anions via Superoxide Dismutase.

ASSAY PRINCIPLE

The DetectX® Superoxide Dismutase (SOD) Activity Kit is designed to quantitatively measure SOD activity in a variety of samples. The assay measures all types of SOD activity, including Cu/Zn-SOD, Mn-SOD, and Fe-SOD types. Please read the complete kit insert before performing this assay.

A bovine erythrocyte SOD standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Samples are diluted in Assay Buffer and added to the wells. The substrate is then added, followed by xanthine oxidase, and the assay is incubated at room temperature for 20 minutes. The xanthine oxidase generates superoxide in the presence of oxygen, which converts the substrate into a yellow-colored product. The plate is then read at 450 nm. As the SOD activity levels increase in the sample, the superoxide concentration and yellow product decreases. The activity of the SOD in the sample is calculated after making a suitable correction for any dilution, using software available with most plate readers. The results are expressed in terms of units of SOD activity per mL.

REAGENT PREPARATION

Except for the reagents listed below, all kit components are ready for use.

Reagent	Preparation	Storage
1X Xanthine Oxidase	Vortex the suspension of 25X Xanthine Oxidase Concentrate prior to pipetting. Pipet from the base of the tube. Mix 1 volume 25X Xanthine Oxidase Concentrate with 24 volumes Xanthine Oxidase Buffer gently (do not vortex). For one plate, mix 100 µL 25X Concentrate with 2.4 mL of Xanthine Oxidase Buffer. Scale accordingly.	1X Xanthine Oxidase must be **stored in an amber vial or otherwise protected from light.** Use immediately.
1X Substrate	Vortex the vial of 10X Substrate Concentrate prior to pipetting. Mix 1 volume 10X Substrate Concentrate with 9 volumes Substrate Diluent gently (do not vortex). For one plate, mix 500 µL 10X Concentrate with 4.5 mL Substrate Diluent. Scale accordingly.	Substrate Diluent must be kept tightly capped. 1X Substrate must be kept **tightly capped and stored in an amber vial or otherwise protected from light.** Use immediately.

SAMPLE PREPARATION

Upon collection, all samples should be frozen rapidly and stored at -80˚C until sample preparation. During sample preparation, samples should be kept on ice to maintain enzyme activity.

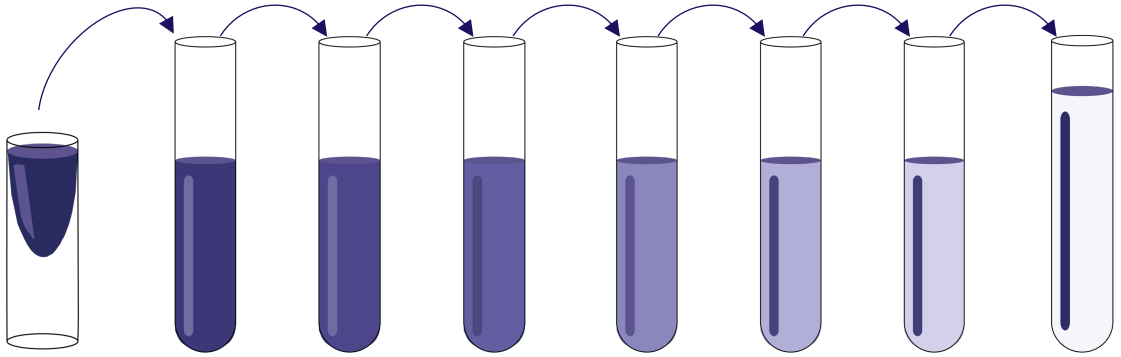
Sample Type	Procedure
Plasma and Serum*	Collect blood in heparin or EDTA tubes (plasma) or serum tubes (serum). Centrifuge the sample at 700 x g for 15 minutes at 4°C. Aspirate and collect the supernatant. Assay immediately or freeze at -80°C. Plasma and serum should be diluted at least 1:5 by mixing 1 volume sample with 4 volumes Assay Buffer prior to assaying. Samples may require further dilution with Assay Buffer to fall within the standard curve range.
Red Blood Cell (RBC) Lysates*	Lyse RBCs by adding 4 volumes of ice-cold deionized water to the pelleted RBCs from the Plasma step above. Centrifuge at 10,000 x g for 15 minutes at 4°C to remove debris. Assay immediately or store at -80°C. Lysed RBCs should be diluted at least 1:100 by mixing 10 µL lysed RBCs with 990 mL Assay Buffer prior to assaying. Samples may require further dilution with Assay Buffer to fall within the standard curve range.
Cell Suspensions, Adherent Cells, And Tissue	• If starting from tissue, wash thoroughly with ice-cold 1X PBS. Proceed to Step 2B. • If starting from adherent cells, wash 1 x 10^6 adherent cells with 1X PBS before harvesting with gentle trypsinization. Proceed to Step 1. 1. Centrifuge ≥ 1 x 10^6 cells at 250 x g for 10 minutes at 4°C. Discard supernatant. 2. A) **Cells**: Resuspend the cell pellet or tissue in 0.5 – 1 mL ice-cold 1X PBS (+ protease inhibitors if desired) per 100 mg of cells and transfer to a microfuge tube. Proceed to step 3 or place on ice. B) **Tissue**: Add 0.5 – 1 mL ice-cold 1X PBS (+ protease inhibitors if desired) per 100 mg of tissue in a microfuge tube. Proceed to step 3 or place on ice. 3. Homogenize or sonicate. 4. Centrifuge at 1,500 x g for 10 minutes at 4°C. Collect the supernatant. 5. Assay the supernatant immediately or store at -80°C. 6. Dilute the supernatant at least 1:4 by mixing one volume supernatant with 3 volumes Assay Buffer prior to assaying. Samples may require further dilution with Assay Buffer to fall within the standard curve range. **OR** To measure cytosolic (Cu/Zn-SOD) and/or mitochondrial SOD (Mn-SOD) the supernatant from Step 1 should be centrifuged at 10,000 x g for 15 minutes at 4°C. The resulting supernatant will contain Cu/Zn-SOD and the cell pellet will contain Mn-SOD. To determine Mn-SOD activity, homogenize the pellet in ice-cold 1X PBS containing a final concentration of 2 mM potassium cyanide. Addition of cyanide will inactivate Cu/Zn SOD enzymes. Assay immediately or store at -80°C.

* Some samples may contain significant amounts of hemoglobin. After adding the Substrate solution to all wells, read and record the optical density at 450 nm. Subtract this measurement from the optical density recorded at the end of the 20-minute assay incubation.

⚠ **Use all samples within 2 hours of dilution.**

STANDARD PREPARATION

1. Label tubes Standard 1 through Standard 7.
2. Add 990 µL Assay Buffer to Standard 1 tube.
3. Add 100 µL Assay Buffer to Standard 2 – 7 tubes.
4. Add 10 µL of Superoxide Dismutase Standard stock solution to Standard 1 tube. Vortex thoroughly.
5. Transfer 100 µL of Standard 1 into Standard 2 tube to make a 2-fold dilution. Vortex thoroughly.
6. Transfer 100 µL of Standard 2 into Standard 3 tube to make a 2-fold dilution. Vortex thoroughly.
7. Continue serially diluting into the remaining tubes. This process and the final concentrations are summarized in the table below.



	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
Assay Buffer (µL)	990	100	100	100	100	100	100
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Volume of Addition (µL)	10	100	100	100	100	100	100
Final Activity (U/mL)	92	46	23	11.5	5.8	2.9	1.4

⚠ **Use all Standards within 2 hours of dilution.**

ASSAY PROTOCOL

Before You Begin:

- **Dilute samples as described in Sample Preparation.**
- **Run all standards and samples in duplicate.**
- Use the blank plate template on the back page of this booklet to design your plate layout and aid in proper sample and standard identification.
- Set plate parameters on the plate reader for a 96-well Corning CoStar 3695 plate. See ArborAssays.com for plate dimension data.

1. Add 10 µL Samples or Standards into duplicate wells in the plate.
2. Add 10 µL Assay Buffer into duplicate wells as the Zero Standard.
3. Add 50 µL 1X Substrate to each well.
   ⚠ If your samples have significant yellow coloration, pre-read the optical density at 450 nm.
4. Gently mix the 1X Xanthine Oxidase and add 25 µL to each well.
   - The solution will begin to turn yellow
5. Incubate at room temperature for 20 minutes.
6. Read the optical density at 450 nm.

CALCULATION OF RESULTS

Follow the instructions below or use the online tool: https://www.myassays.com/assay.aspx?id=968

1. Use four-parameter logistic curve (4PLC) software to calculate the SOD activity for each sample. Gather all raw data OD readings from each Sample and Standard, including the Zero Standard.
2. Average the duplicate OD readings for each Sample, Standard, and Zero Standard (Mean OD).

EXAMPLE:

Sample	Replicate 1 OD	Replicate 2 OD	Mean OD
Standard 1	0.120	0.128	0.124
Sample 1	0.341	0.343	0.342
Sample 2	0.463	0.469	0.466
Sample 3	0.822	0.834	0.828

3. If the OD was pre-read before incubation (Pre-Incubation OD), subtract this Pre-Incubation OD from the Mean OD and proceed with this Corrected Mean OD.

EXAMPLE:

Sample	Mean OD	Pre-Incubation OD	Corrected Mean OD
Standard 1	0.124	n/a	
Sample 1	0.342	n/a	
Sample 2	0.466	n/a	
Sample 3	0.828	0.095	0.733

4. Plot the standard curve with Mean OD for the Standards on the y-axis and SOD activity (U/mL) on the x-axis. Perform a 4PLC fit.

   Use the sample Mean OD (or Corrected Mean OD) readings and the 4PLC fit to calculate SOD activity in diluted samples. If diluted sample SOD activity is outside of the range of the standards, the sample should be prepared again at a more appropriate dilution.

EXAMPLE:

Sample	Mean (Corrected) OD	SOD Activity (U/mL)
Sample 1	0.342	23.7
Sample 2	0.466	13.5
Sample 3	(0.733)	2.9

5. If the original sample was diluted, multiply the sample SOD activity by the sample dilution factor to determine the activity of SOD in the original sample.

EXAMPLE:

Sample	SOD Activity (U/mL)	Sample Modification Factor	Original Sample SOD Activity (U/mL)
Sample 1	23.7	n/a	23.7
Sample 2	13.5	4	54.0
Sample 3	2.9	5	14.5

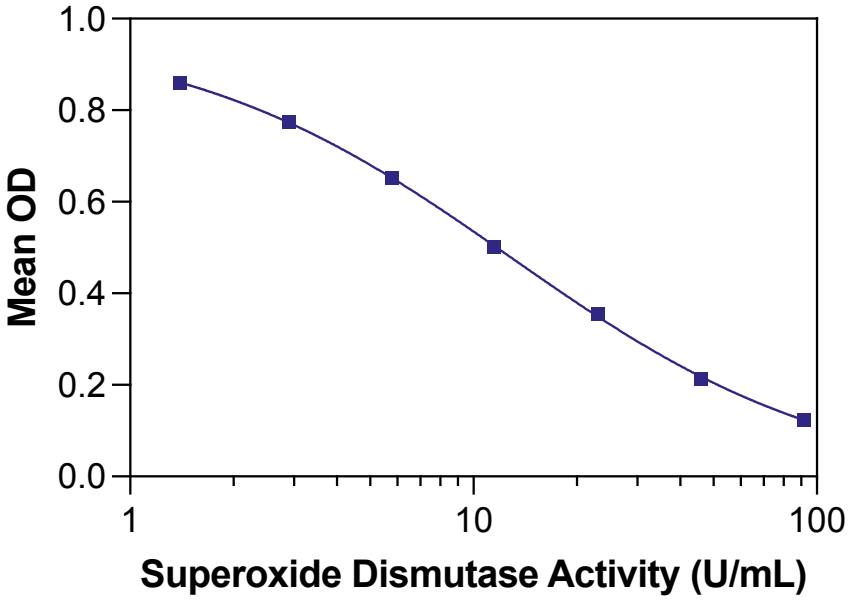
TYPICAL DATA

⚠ **Always run your own standard curve. This data should NOT be used to interpret results.**

Sample	Mean OD	SOD Activity (U/mL)
Standard 1	0.124	92.0
Standard 2	0.214	46.0
Standard 3	0.355	23.0
Standard 4	0.500	11.5
Standard 5	0.652	5.8
Standard 6	0.774	2.9
Standard 7	0.860	1.4
Zero	0.939	0.0
Sample 1	0.342	23.7
Sample 2	0.466	13.5

SOD Unit Definition
One unit of SOD is defined as the amount of enzyme causing half the maximum inhibition of the oxidation of 7.5 mM NADH in the presence of EDTA, manganese ions, and mercaptoethanol at 23°C and pH 7.4 over 15 minutes[12].

Typical Standard Curve



VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the ODs for twenty wells run for each of the Zero Standard and Standard 7. The detection limit was determined at two standard deviations from the zero along the standard curve.

Sensitivity was determined as 0.422 U/mL.

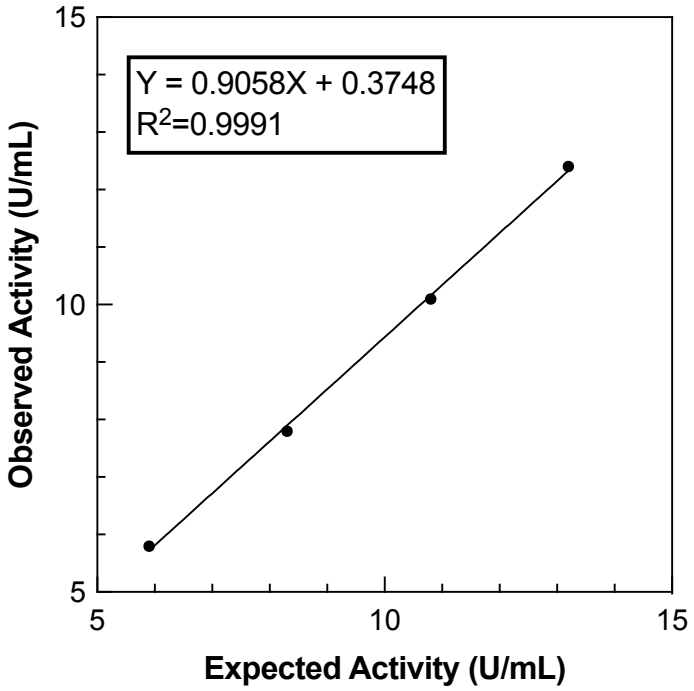
The Limit of Detection was determined in a similar manner by comparing the ODs for twenty wells run for each of the Zero Standard and a low concentration sample.

The Limit of Detection was determined as 0.200 U/mL.

Linearity

Linearity was determined in red blood cell lysates by diluting two samples with known SOD activity in Assay Buffer. One sample had a SOD activity of 3.4 U/mL (Low Sample), and a second had a SOD activity of 15.7 U/mL (High Sample). The two samples were mixed in the ratios given below and the measured concentrations were compared to the expected values for each given ratio.

Low Sample	High Sample	Expected Activity (U/mL)	Observed Activity (U/mL)	% Recovery
80%	20%	5.9	5.8	99.0
60%	40%	8.3	7.8	93.8
40%	60%	10.8	10.1	93.7
20%	80%	13.2	12.4	93.7
			Mean Recovery	**95.0%**



Intra Assay and Inter Assay Precision

For intra assay precision, four samples were diluted in Assay Buffer and were run in replicates of 20 in one assay. For inter assay precision, four samples were diluted in Assay Buffer and duplicates of each sample were run in 20 assays over multiple days by five operators. The %CV represents the variation in activity (not optical density) as determined using a standard curve.

	Intra Assay Precision		Inter Assay Precision	
Sample	SOD Activity (U/mL)	% CV	SOD Activity (U/mL)	% CV
1	25.5	2.0	23.7	8.3
2	20.7	2.5	19.3	9.2
3	15.5	3.0	13.5	10.9
4	7.4	1.4	6.6	15.3

SAMPLE VALUES

10 human serum and 10 human EDTA plasma samples were diluted in Assay Buffer and run in the assay. Five samples of Red Blood Cells (RBCs) from EDTA plasma were normalized to hemoglobin (Hgb) levels using the DetectX® Hemoglobin Detection kit, K013-H1. The average activities and ranges for each sample type are shown below.

Sample Type	Recommended Minimum Dilution	Adjusted Average Concentration	Adjusted Average Concentration Range
Serum	1:5	11.5 U/mL	7.3 – 14.4 U/mL
Plasma	1:5	13.8 U/mL	8.8 – 19.1 U/mL
RBCs	1:100	42,120 U/g Hgb	34,718 – 50,943 U/g Hgb

INTERFERENCE

Bilirubin and hemoglobin were evaluated at high and low concentrations of SOD to evaluate their potential to interfere with the assay.

Interferent	Effect at High SOD Concentration	Effect at Low SOD Concentration
Bilirubin (5 mg/dL)	1.7% Decrease in Signal	7.9% Decrease in Signal
Hemoglobin (0.4 mg/dL)	8.8% Decrease in Signal	6.9% Decrease in Signal

TROUBLESHOOTING

Issue	Possible Cause & Solution
Reagent Shortage	• Check under the cap for additional reagent. Pulse spin reagent containers to collect contents prior to opening when possible. • When using a multichannel pipette, return unused reagent to container for later use.
Erratic Values	• Prerinse pipet tips with desired reagent prior to aspirating the required volume. • Deliver volume with care to prevent splashing into adjacent wells. • Preparation and dispensing of 1X Xanthine Oxidase.
Low Signal	• Verify the plate reader is set to 450 nm. • Confirm reagents are at room temperature prior to use.
Legacy Results	• To improve the assay robustness, the definition of one unit of SOD was redefined on July 22, 2024 (see page 10). To compare results after this date with results obtained previously, multiply the previous activity results by 23. • The previous definition of one unit of SOD was the amount of enzyme causing half the maximum inhibition of the reduction of 1.5 mM nitro blue tetrazolium (NBT) in the presence of riboflavin at 25°C and pH 7.8.

CITATIONS

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RELATED PRODUCTS

Kits	Catalog No.
Catalase Colorimetric Detection Kit	K033-H1
Catalase Fluorescent Detection Kit	K033-F1
Glutathione Colorimetric Detection Kit	K006-H1
Glutathione Fluorescent Detection Kits	K006-F1/F5
Glutathione Reductase Fluorescent Activity Kit	K009-F1
Hemoglobin Dual Range Detection Kit	K013-H1
Hydrogen Peroxide Colorimetric Activity Kit	K034-H1
Hydrogen Peroxide Fluorescent Activity Kit	K034-F1
Nitric Oxide Colorimetric Detection Kit	K024-H1

LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us.

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with assay kits and reagents for wildlife conservation research.

PLATE LAYOUT

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