DetectX®

Testosterone Enzyme Immunoassay Kit

1 or 5 Strip Plates  Catalog Number K032-H1/H5
1 or 5 Whole Plates  Catalog Number K032-H1W/H5W

Species Independent

<table>
<thead>
<tr>
<th>Sample Types Validated:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried Fecal Extracts, Urine, Extracted Serum/Plasma, and Tissue Culture Media</td>
</tr>
</tbody>
</table>

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

www.ArborAssays.com  

WEB K023-H 231207
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BACKGROUND

Testosterone, \( \text{C}_{19}\text{H}_{28}\text{O}_{2} \), (4-Androsten-17\(\beta\)-ol-3-one) is a steroid hormone from the androgen group and is found in mammals, reptiles, birds, and other vertebrates\(^1\). In mammals, testosterone is primarily secreted in the testes of males and the ovaries of females, although small amounts are also secreted by the adrenal glands. It is the principal male sex hormone and an anabolic steroid.

In men, testosterone plays a key role in the development of male reproductive tissues such as the testis and prostate as well as promoting secondary sexual characteristics such as increased muscle, bone mass, and the growth of body hair\(^3\). In the absence of testosterone stimulation, spermatogenesis does not proceed beyond the meiosis stage. In addition, testosterone is essential for health and well-being\(^4\) as well as the prevention of osteoporosis\(^5\). On average, an adult human male body produces about ten times more testosterone than an adult human female body, but females are more sensitive to the hormone\(^6\). Testosterone plays a significant role in glucose homeostasis and lipid metabolism. The metabolic syndrome is a clustering of risk factors predisposing to type 2 diabetes mellitus, atherosclerosis and cardiovascular morbidity and mortality. The main components of the syndrome are visceral obesity, insulin resistance, glucose intolerance, raised blood pressure and dyslipidemia (elevated triglycerides, low levels of high-density lipoprotein cholesterol), and a pro-inflammatory and thrombogenic state. Cross-sectional epidemiological studies have reported a direct correlation between plasma testosterone and insulin sensitivity, and low testosterone levels are associated with an increased risk of type 2 diabetes, dramatically illustrated by androgen deprivation in men with prostate carcinoma.

Testosterone is observed in most vertebrates. Fish make a slightly different form called 11-ketotestosterone\(^7\). Its counterpart in insects is ecdysone\(^8\) These ubiquitous steroids suggest that sex hormones have an ancient evolutionary history\(^9\).

ASSAY PRINCIPLE

The DetectX® Testosterone Immunoassay Kit uses a specifically generated antibody to measure testosterone and its metabolites in urine and fecal samples, or in extracted serum and plasma. This kit is not recommended for serum, plasma, or saliva samples without extraction. The kit will quantitatively measure Testosterone present in reconstituted buffer samples and tissue culture media samples. Please read the complete kit insert before performing this assay. A testosterone standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture rabbit antibodies. A testosterone-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a polyclonal antibody to testosterone to each well. After a 2 hour incubation the plate is washed and substrate is added. The substrate reacts with the bound testosterone-peroxidase conjugate. After a short incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450 nm wavelength. The concentration of the testosterone in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.

RELATED PRODUCTS

<table>
<thead>
<tr>
<th>Kits</th>
<th>Catalog No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androstenedione ELISA Kits</td>
<td>K070-H1/H5</td>
</tr>
<tr>
<td>Corticosterone ELISA Kits</td>
<td>K014-H1/H5/H1W/H5W</td>
</tr>
<tr>
<td>Cortisol Enzyme Immunoassay Kits (Strip Wells and Whole Plate)</td>
<td>K003-H1/H5/H1W/H5W</td>
</tr>
<tr>
<td>Cortisone ELISA and Chemiluminescent ELISA Kits</td>
<td>K017-H1/H5, K017-C1/C5</td>
</tr>
<tr>
<td>Dehydro-epiandrosterone sulfate (DHEA-S) ELISA Kits</td>
<td>K054-H1/H5</td>
</tr>
<tr>
<td>Epiandrosterone ELISA Kits</td>
<td>K063-H1/H5</td>
</tr>
<tr>
<td>Estradiol Non-Invasive &amp; Serum ELISA Kits</td>
<td>K030-H1/H5, KB30-H1/H5</td>
</tr>
<tr>
<td>Estrone ELISA Kits</td>
<td>K031-H1/H5</td>
</tr>
<tr>
<td>PGFM (13,14-dihydro-15-keto-Prostaglandin F2alpha) ELISA Kits</td>
<td>K022-H1/H5</td>
</tr>
<tr>
<td>Pregnanediol 3-Glucuronide (PDG) ELISA Kits</td>
<td>K037-H1/H5</td>
</tr>
<tr>
<td>Progesterone Metabolites ELISA Kits</td>
<td>K068-H1/H5</td>
</tr>
<tr>
<td>Urinary Creatinine Detection Kits</td>
<td>K002-H1/H5</td>
</tr>
</tbody>
</table>
SUPPLIED COMPONENTS

Coated Clear 96 Well Plates
Clear plastic microtiter plate(s) coated with goat anti-rabbit IgG.
Kit K032-H1 or H5 1 or 5 Each  Catalog Number X016-1EA, 1 x 8 Strip Well
Kit K032-H1W or H5W 1 or 5 Each  Catalog Number X015-1EA, Whole Well

Testosterone Standard
Testosterone at 200,000 pg/mL in a special stabilizing solution.
Kit K032-H1/H1W or -H5/H5W 70 µL or 350 µL Catalog Number C113-70UL or -350UL

DetectX® Testosterone Antibody
A rabbit polyclonal antibody specific for testosterone
Kit K032-H1/H1W or -H5/H5W 3 mL or 13 mL Catalog Number C111-3ML or -13ML

DetectX® Testosterone Conjugate  Must be stored at ≤ -20°C.
A testosterone-peroxidase conjugate in a special stabilizing solution.
Kit K032-H1/H1W or -H5/H5W 3 mL or 13 mL Catalog Number C315-3ML or -13ML

Assay Buffer Concentrate
A 5X concentrate that should be diluted with deionized or distilled water.
Kit K032-H1/H1W or -H5/H5W 28 mL or 55 mL Catalog Number X065-28ML or -55ML

Wash Buffer Concentrate
A 20X concentrate that should be diluted with deionized or distilled water.
Kit K032-H1/H1W or -H5/H5W 30 mL or 125 mL Catalog Number X007-30ML or -125ML

TMB Substrate
Kit K032-H1/H1W or -H5/H5W 11 mL or 55 mL Catalog Number X019-11ML or -55ML

Stop Solution
A 1M solution of hydrochloric acid. CAUSTIC.
Kit K032-H1/H1W or -H5/H5W 5 mL or 25 mL Catalog Number X020-5ML or -25ML

Plate Sealer
Kit K032-H1/H1W or -H5/H5W 1 or 5 Each  Catalog Number X002-1EA

STORAGE INSTRUCTIONS

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 Testosterone Conjugate. This must be stored at ≤ -20°C.
OTHER MATERIALS REQUIRED

Distilled or deionized water.
Polypropylene or glass test tubes.
Repeater pipet with disposable tips capable of dispensing 25, 50, and 100 μL.
Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.
Diethyl ether or ethyl acetate for extraction of serum and plasma.
Ethanol for extraction of fecal material.
A Speedvac/centrifugal concentrator or N₂ gas and gas manifold for evaporation.
Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The testosterone standard used for this kit is an anabolic steroid and may have a number of known and unknown biological actions. Care should be taken in handling this material.

The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers’ Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure all buffers used for samples are azide free. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 8.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.
SAMPLE TYPES

This assay has been validated for dried fecal, urine and for tissue culture samples. Samples containing visible particulate should be centrifuged prior to using. Testosterone can be assayed in solid sample types or in serum and plasma samples by using one of the extraction protocols available on our website at: www.ArborAssays.com/resources/#protocols

Testosterone is identical across all species and we expect this kit to measure testosterone from all sources. The end user should evaluate recoveries of testosterone in other sample matrices being tested.

SAMPLE PREPARATION

Serum and Plasma Samples
We have 3 detailed Extraction Protocols available on our website at: www.ArborAssays.com/resources/#protocols as a PDF file entitled “Steroid Serum/Plasma Extraction Protocol”. We would recommend the following protocol for serum and plasma.

1. Add diethyl ether to serum or plasma samples at a 5:1 (v/v) ether:sample ratio.
2. Mix solutions by vortexing for 2 minutes. Allow ether layer to separate for 5 minutes.
3. Freeze samples in a dry ice/ethanol bath and pipet off the ether solution from the top of the sample into a clean tube. Repeat steps 1-3 for maximum extraction efficiency, combining top layer of ether solutions.
4. Dry pooled ether samples down in a speedvac for 2-3 hrs. If samples need to be stored they should be kept at -20°C.
5. Redissolve samples at room temperature in the Assay Buffer. A minimum of 125 μL of the Assay Buffer is recommended for reconstitution to allow for duplicate sample measurement.

Dried Fecal Samples
We have a detailed Extraction Protocol available on our website at: www.ArborAssays.com/resources/#protocols. The ethanol concentration in the final Assay Buffer dilution added to the well should be < 5%.

Urine Samples
Urine samples should be diluted at least 1:4 with the provided Assay Buffer. For comparison to creatinine as a urine volume marker please see our NIST-calibrated Urinary Creatinine Detection Kits, K002-H1 and K002-H5.

Tissue Culture Media
For measuring testosterone in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. We have validated the assay using RPMI-1640.

Use all samples within 2 hours of preparation.
REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

**Assay Buffer**
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

**Wash Buffer**
Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at room temperature for 3 months.

**Standard Preparation**
Label test tubes as #1 through #7. Pipet 475 µL of Assay Buffer into tube #1 and 300 µL into tubes #2 to #7. The testosterone stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 25 µL of the testosterone stock solution to tube #1 and vortex completely. Take 200 µL of the testosterone solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #7. The concentration of testosterone in tubes 1 through 7 will be 10,000, 4,000, 1,600, 640, 256, 102.4 and 40.96 pg/mL.

Use all Standards within 2 hours of preparation.

<table>
<thead>
<tr>
<th></th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std 5</th>
<th>Std 6</th>
<th>Std 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer (µL)</td>
<td>475</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Addition</td>
<td>Stock</td>
<td>Std 1</td>
<td>Std 2</td>
<td>Std 3</td>
<td>Std 4</td>
<td>Std 5</td>
<td>Std 6</td>
</tr>
<tr>
<td>Vol of Addition (µL)</td>
<td>25</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Final Conc (pg/mL)</td>
<td>10,000</td>
<td>4,000</td>
<td>1,600</td>
<td>640</td>
<td>256</td>
<td>102.4</td>
<td>40.96</td>
</tr>
</tbody>
</table>
ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine testosterone concentrations.

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification.

2. If you are using the 1 by 8 well strip plate version of the kit, K032-H1 or -H5, determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.

   Pipet standards or samples down the plate strip columns (A to H) to ensure maximum use of the strip wells.

   The use of any wells in the whole plate versions of the kit, K032-H1W and K032-H5W will not allow use of unused parts of that plate in a later assay.

3. Pipet 50 µL of samples or standards into wells in the plate.

4. Pipet 75 µL of Assay Buffer into the non-specific binding (NSB) wells.

5. Pipet 50 µL of Assay Buffer into the maximum binding (B0 or Zero standard) wells.

6. Add 25 µL of the DetectX® Testosterone Conjugate to each well using a repeater pipet.

7. Add 25 µL of the DetectX® Testosterone Antibody to each well, except the NSB wells, using a repeater pipet.

8. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 2 hours. We recommend shaking at around 700–900 rpm. If the plate is not shaken, signals bound will be approximately 20% lower.

9. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.

10. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.

11. Incubate the plate at room temperature for 30 minutes without shaking.

12. Add 50 µL of the Stop Solution to each well, using a repeater pipet.

13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.

14. Use the plate reader’s built-in 4PLC software capabilities to calculate testosterone concentration for each sample.

NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.
CALCULATION OF RESULTS

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD’s for the NSB. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-testosterone-eia-kit.assay

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean OD</th>
<th>Net OD</th>
<th>% B/B0</th>
<th>Testosterone Conc. (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSB</td>
<td>0.076</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 1</td>
<td>0.248</td>
<td>0.172</td>
<td>10.5</td>
<td>10,000</td>
</tr>
<tr>
<td>Standard 2</td>
<td>0.389</td>
<td>0.313</td>
<td>19.2</td>
<td>4,000</td>
</tr>
<tr>
<td>Standard 3</td>
<td>0.576</td>
<td>0.500</td>
<td>30.7</td>
<td>1,600</td>
</tr>
<tr>
<td>Standard 4</td>
<td>0.828</td>
<td>0.752</td>
<td>46.1</td>
<td>640</td>
</tr>
<tr>
<td>Standard 5</td>
<td>1.113</td>
<td>1.037</td>
<td>63.6</td>
<td>256</td>
</tr>
<tr>
<td>Standard 6</td>
<td>1.358</td>
<td>1.282</td>
<td>78.6</td>
<td>102.4</td>
</tr>
<tr>
<td>Standard 7</td>
<td>1.495</td>
<td>1.419</td>
<td>87.0</td>
<td>40.96</td>
</tr>
<tr>
<td>B0</td>
<td>1.707</td>
<td>1.631</td>
<td>100.0</td>
<td>0</td>
</tr>
<tr>
<td>Sample 1</td>
<td>0.595</td>
<td>0.519</td>
<td>31.8</td>
<td>1478</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.737</td>
<td>0.661</td>
<td>40.5</td>
<td>849</td>
</tr>
<tr>
<td>Sample 3</td>
<td>0.962</td>
<td>0.886</td>
<td>54.3</td>
<td>406</td>
</tr>
<tr>
<td>Sample 4</td>
<td>1.076</td>
<td>1.000</td>
<td>61.3</td>
<td>262</td>
</tr>
</tbody>
</table>

Always run your own standard curve for calculation of results. Do not use this data.
Conversion Factor: 100 pg/mL of testosterone is equivalent to 346.7 pM.
Always run your own standard curves for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity and Limit of Detection
Sensitivity was calculated by comparing the OD’s for twenty wells run for each of the B0 and standard #7. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve. Sensitivity was determined as 15.2 pg/mL.

The Limit of Detection for the assay was determined in a similar manner by comparing the OD’s for twenty runs for each of the zero standard and a low concentration human urine sample. Limit of Detection was determined as 30.6 pg/mL.
**Linearity**

Linearity was determined by taking two urine samples diluted with Assay Buffer, one with a low diluted testosterone level of 119.9 pg/mL and one with a higher diluted level of 765.6 pg/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

<table>
<thead>
<tr>
<th>High Urine</th>
<th>Low Urine</th>
<th>Expected Conc. (pg/mL)</th>
<th>Observed Conc. (pg/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>20%</td>
<td>636.4</td>
<td>657.9</td>
<td>103.4</td>
</tr>
<tr>
<td>60%</td>
<td>40%</td>
<td>507.3</td>
<td>508.6</td>
<td>100.3</td>
</tr>
<tr>
<td>40%</td>
<td>60%</td>
<td>378.2</td>
<td>435.1</td>
<td>115.1</td>
</tr>
<tr>
<td>20%</td>
<td>80%</td>
<td>249.0</td>
<td>278.3</td>
<td>111.7</td>
</tr>
</tbody>
</table>

Mean Recovery 107.6%

**Linearity**

\[ y = 0.9389x + 54.299 \]

\[ R^2 = 0.9829 \]
**Intra Assay Precision**
Three human samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated Testosterone concentrations were:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Testosterone Conc. (pg/mL)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,384</td>
<td>5.7</td>
</tr>
<tr>
<td>2</td>
<td>275.8</td>
<td>11.1</td>
</tr>
<tr>
<td>3</td>
<td>147.5</td>
<td>15.8</td>
</tr>
</tbody>
</table>

**Inter Assay Precision**
Four human samples were diluted with Assay Buffer and run in duplicates in ten assays run over multiple days by five operators. The mean and precision of the calculated Testosterone concentrations were:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Testosterone Conc. (pg/mL)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,478</td>
<td>7.1</td>
</tr>
<tr>
<td>2</td>
<td>849</td>
<td>14.2</td>
</tr>
<tr>
<td>3</td>
<td>406</td>
<td>17.7</td>
</tr>
<tr>
<td>4</td>
<td>262</td>
<td>12.9</td>
</tr>
</tbody>
</table>
SAMPLE VALUES

Eleven urine samples from various species were tested in the assay. Adjusted neat concentrations of Testosterone ranged from 2.331 to 215.0 ng/mL. When adjusted for urine creatinine using the DetectX® Urinary Creatinine Detection kit, K002-H1, the values ranged from 16.2 to 1,567 ng/mg creatinine.

Fecal samples from Dune, a male fennec fox, were extracted and tested in the assay.

The fennec fox sample was the kind gift from Jocelyn Bryant, Brookfield Zoo, Brookfield, IL.

CROSS REACTIVITY

The following cross reactants were tested in the assay and calculated at the 50% binding point.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Cross Reactivity (%)</th>
<th>Steroid</th>
<th>Cross Reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>100%</td>
<td>Pregnenolone</td>
<td>&lt; 0.02%</td>
</tr>
<tr>
<td>5α-Dihydrotestosterone</td>
<td>56.8%</td>
<td>Hydrocortisone</td>
<td>&lt; 0.02%</td>
</tr>
<tr>
<td>11-Ketotestosterone</td>
<td>2.34%</td>
<td>Cholic Acid</td>
<td>&lt; 0.02%</td>
</tr>
<tr>
<td>Androstendione</td>
<td>0.27%</td>
<td>Cholic Derivatives</td>
<td>&lt; 0.02%</td>
</tr>
<tr>
<td>Androsterone</td>
<td>0.04%</td>
<td>Aldosterone</td>
<td>&lt; 0.02%</td>
</tr>
<tr>
<td>DHEA</td>
<td>0.04%</td>
<td>Cortisol</td>
<td>&lt; 0.02%</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.03%</td>
<td>Corticosterone</td>
<td>&lt; 0.02%</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>0.02%</td>
<td>Cortisone</td>
<td>&lt; 0.02%</td>
</tr>
<tr>
<td>Progesterone</td>
<td>&lt; 0.02%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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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Fax: 734-677-6860
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Technical@ArborAssays.com
Contracts@ArborAssays.com

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 ELISA kits for wildlife conservation research.

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