Birth Defects and Folic Acid
Teacher Information

Summary
Students follow the case of a pregnant woman who is concerned that her developing baby is at risk for spina bifida, a birth defect that results from an incomplete closing of the spinal cord or its coverings. They test the mother’s blood and amniotic fluid for alpha-fetoprotein (AFP) and interpret the results of a fetal sonogram. They explore the importance of prenatal care for preventing birth defects.

Core Concepts
• The development of the nervous system begins early in a pregnancy.
• Exposure to or lack of specific chemicals can interfere with the development of the nervous system.
• Prenatal testing may indicate abnormalities in the development process.

Time Required
Three 40-minute class periods for activities. Allow two additional class periods if students complete the optional Internet search and brochure to report on other birth defects.

Kit contains
• Tube of Anita’s Blood Serum
• AFP Fast-Test Strip
• Tube of Amniotic Fluid
• Dropper labeled Amniotic Fluid
• AFP Test Sheet
• Tube of AFP Indicator
• Dropper for AFP Indicator
• AFP Color Chart
• “Ectoderm” (clay)
• Neural Tube Defects Fact Sheet
• Ultrasound and Amniocentesis information sheet
• Two cards—Experimental Group and Control Group
• Penny
• Preventing Birth Defects brochure

Teacher Provides
• Safety goggles
• Paper towels for clean up

Warning: Choking Hazard
This Science Take-Out kit contains small parts. Do not allow children under the age of seven to have access to any kit components.
Teacher Notes

- Be aware that some of your students may have spina bifida. Spina bifida can be mild or severe.
  - The mild form does not cause problems or need treatment.
  - The severe form of spina bifida results when spinal nerves push out of the spinal canal causing nerve damage that leads to problems with walking, bladder or bowel control, and coordination.

Optional Extension Activity: Exploring Birth Defects

Do an Internet search to identify at least four additional birth defects that affect the nervous system. Hint: Use the search terms “birth defect nervous system” and “birth defect brain.”

Select ONE birth defect that affects the nervous system. Prepare an illustrated poster or brochure about the birth defect. Your poster should provide the following information.

- Symptoms (What would it be like to have this birth defect?)
- Cause (be specific if the cause is known)
- Diagnosis (What tests can be done to determine if a baby has this defect—both before and after birth?)
- Treatment (What can be done to relieve symptoms?)
- Prevention
- Other information you found interesting (at least three things)
- What could you do to help someone with this birth defect enjoy a more normal life?
Reusing *Birth Defects and Folic Acid* kits

Teachers will need to instruct students on how to handle clean-up and return of the re-usable kit materials. For example, teachers might provide the following information for students:

<table>
<thead>
<tr>
<th>Discard</th>
<th>Rinse with water</th>
<th>Return to kit bag</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Used AFP Fast–Test Strips</td>
<td>• AFP test sheet</td>
<td>• All labeled droppers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• All labeled tubes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• AFP Fast–Test bag</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• AFP Color Chart</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• AFP test sheet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Ectoderm (clay)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Neural Tube Defects fact sheet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Experimental and Control Group Cards</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Preventing Birth Defects Brochure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Ultrasound and Amniocentesis information sheet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Penny</td>
</tr>
</tbody>
</table>

**Note:** It is **not** necessary to rinse or wash the droppers after use. Because the droppers are labeled, there is little chance for contamination. Washing the droppers may make the labels difficult to read. Simply ask students to squirt out any extra liquid from the droppers.

Consider laminating printed parts of the kits (such as colored graphics or instruction cards) that will be reused.

Refills for *Birth Defects and Folic Acid* kits are available at www.sciencetakeout.com. The 10 Kit Refill Pack includes the following materials:

- Instructions and Quick Guide for refilling kit
- 3 graduated transfer pipets (for teacher use)
- 10 AFP Test Strips
- 15 ml of “Anita’s Blood Serum”
- 5 ml of “Amniotic Fluid”
- 5 ml of “AFP Indicator”
Kit Contents Quick Guide

AFP Test Sheet
2 drops of amniotic fluid
2 drops of AFP Indicator

Fact Sheet: NEURAL TUBE DEFECTS

Fact Sheet: NEURAL TUBE DEFECTS

Preventing Birth Defects
What is a Birth Defect?
A birth defect is an abnormal condition that occurs before or at the time of birth. Birth defects can cause physical and mental problems for both the mother and baby. The causes of birth defects are varied and can include genetic factors, environmental factors, and maternal health issues. Prevention measures, such as prenatal care, can help reduce the risk of birth defects.

Control Group

Experimental Group

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www.sciencetakeout.com
Read these instructions before using Science Take-Out kits

Parental or Adult Supervision Required
This kit should be used only under the supervision of an adult who is committed to ensuring that the safety precautions below, and in the specific laboratory activity, are followed.

Safety Goggles and Gloves Strongly Recommended
We encourage students to adopt safe lab practices, and wear safety goggles and gloves when performing laboratory activities involving chemicals. Safety goggles and gloves are not provided in Science Take-Out kits. They may be purchased from a local hardware store or pharmacy.

Warning: Choking and Chemical Hazard
Science Take–Out kits contain small parts that could pose a choking hazard and chemicals that could be hazardous if ingested. Do not allow children under the age of seven to have access to any kit components. Material Safety Data Sheets (MSDS) provide specific safety information regarding the chemical contents of the kits. MSDS information for each kit is provided in the accompanying teacher instructions.

Chemicals Used in Science Take–Out Kits
Every effort has been made to reduce the use of hazardous chemicals in Science Take–Out kits. Most kits contain common household chemicals or chemicals that pose little or no risk.

General Safety Precautions
1. Work in a clean, uncluttered area. Cover the work area to protect the work surface.
2. Read and follow all instructions carefully.
3. Pay particular attention to following the specific safety precautions included in the kit activity instructions.
4. Goggles and gloves should be worn while performing experiments using chemicals.
5. Do not use the contents of this kit for any other purpose beyond those described in the kit instructions.
6. Do not leave experiment parts or kits where they could be used inappropriately by others.
7. Never taste or ingest any chemicals provided in the kit – they may be toxic.
8. Do not eat, drink, apply make–up or contact lenses while performing experiments.
9. Wash your hands before and after performing experiments.
10. Chemicals used in Science Take–Out experiments may stain or damage skin, clothing or work surfaces. If spills occur, wash the area immediately and thoroughly.
11. At the end of the experiment, return ALL kit components to the kit plastic bag. Dispose of the plastic bag and contents in your regular household trash.

No blood or body fluids from humans or animals are used in Science Take–Out kits. Chemical mixtures are substituted as simulations of these substances.
Birth Defects and Folic Acid: *Teacher Answer Key*

**Part 1: Patient History**

Anita Chavez, a 30-year-old woman, came to the free prenatal health clinic because she is expecting her second child. Anita already has a daughter who was born with a neural tube defect called spina bifida.

She did not realize that she was pregnant again until her third month of pregnancy. Once she found out she was pregnant, she started taking prenatal vitamins, and she stopped smoking and drinking. She also started being more careful to control her weight and diabetes.

Anita is worried that her next baby will also have spina bifida or a similar birth defect.

Use the information in the *Neural Tube Defects Fact Sheet* to answer the following questions.

1. List four risk factors that may increase Anita’s risk of having a second child with a neural tube defect.

   Her risk factors include: obesity, diabetes, Hispanic background, previous child with neural tube defect, poverty, failure to take prenatal vitamins early in pregnancy, etc.

2. Do these risk factors mean that Anita’s developing baby will definitely be born with a neural tube defect? Explain why or why not.

   No, just because Anita has many risk factors, it doesn’t mean that her baby will definitely have a neural tube defect.

3. Are there actions that Anita could take during the remainder of her pregnancy to ensure that her developing baby will not develop spina bifida or anencephaly?

   According to the information in the fact sheet, neural tube defects occur very early in pregnancy. So it is too late to take preventative action.
Part 2: Testing Anita’s Blood—Alpha-Fetoprotein Test

Anita’s doctor orders an alpha-fetoprotein (AFP) blood test. This test is done to check the level of AFP in a pregnant woman's blood. AFP is a substance made in the liver of an unborn baby. Normally, low levels of AFP can be found in the blood of a pregnant woman. A high level of AFP in the mother’s blood means that her developing baby is more likely to have a birth defect.

However, if a high level of AFP is found, a neural tube defect is present only a small percentage of the time. The AFP test may give a false positive result. A false positive result means that the test may indicate that baby has a problem when it is in fact healthy. High levels of AFP can be caused by other factors — including if there is a miscalculation in fetal age or if the mother is carrying multiple fetuses (twins or more).

1. Follow the instructions below to test the level of alpha-fetoprotein (AFP) in Anita’s Blood Serum (liquid part of blood).
   - Dip the end of the AFP Fast-Test Strip with the black line into Anita’s blood serum as shown on the right. Be certain that at least half of the test strip is dipped into the serum.
   - If a pink line or dot appears on test strip, it indicates that there is a high level of AFP in the mother’s blood plasma.

2. What conclusions can you draw from the results of the AFP test?
   ________ ________ (yes or no)
   Explain your answer with two pieces of evidence.

3. Can you be certain that Anita’s developing baby will be born with a neural tube defect?
   __________________ (yes or no)

   Explain your answer with two pieces of evidence.
Part 3: Further Testing—Ultrasound and Amniocentesis

Anita’s doctor explains that she should go for further testing that will provide more information about her baby’s development. He schedules her for a sonogram and amniocentesis. He gives Anita an information sheet that explains the sonogram and amniocentesis procedures.

Use the information in the *Ultrasound: Sonogram and Amniocentesis* information sheet to answer the following questions.

1. Which procedure is safest for the mother and the developing baby—sonogram or amniocentesis? Explain why.

   A sonogram because a sonogram has no known harmful effects on the mother or unborn child and requires no probing or biological samples to be taken.

2. The sonograms shown below show a normal 13 week fetus and Anita’s fetus. Find the area on the baby’s sonogram that suggests that Anita’s baby has a neural tube defect. Circle this area on the sonogram.

3. What is amniotic fluid?

4. To collect amniotic fluid, a needle is inserted through the mother’s _________ and _________ and through the ________________ that surrounds the fetus.

5. Explain how a doctor knows where to insert the needle so that it draws amniotic fluid from the proper location and does not damage the developing fetus.
6. Your lab kit contains a sample of **Amniotic Fluid** collected from the amnion that surrounds Anita's fetus. Follow these instructions to determine the level of alpha fetoprotein in the sample. Use the labeled droppers provided to:

- Place 2 drops of amniotic fluid in the circle on the AFP Test Sheet.
- Add 2 drops of AFP Indicator to the fluid in the circle on the AFP Test Sheet.
- Use the color AFP Color Chart to determine the level of alpha fetoprotein in the sample.
- Record the AFP level ______

7. An alpha fetoprotein level above 1.5 indicates an increased chance of having a neural tube defect. Do the results of this test indicate that Anita’s fetus definitely has a neural tube defect? Explain your answer.

No, the level of 2.0 means that there is a chance the baby has a neural tube defect but it does not mean the baby will definitely have a neural tube defect.
Part 4: What Happens During Development to Cause a Neural Tube Defect?

The exact cause of neural tube defects remains a mystery. No one knows what disrupts development of the nervous system to cause these birth defects. Scientists suspect genetic, nutritional, and environmental factors play a role.

The human nervous system develops from embryonic tissue called ectoderm. Ectoderm gives rise to the skin, brain, spinal cord, and branching nerves.

Use the clay (ectoderm) in your kit and the diagrams below to model neural tube development.

1. The first sign of the developing nervous system is the thickening of the ectoderm on the surface of the embryo to form a neural plate that can be seen at about the 16th day of development.
   - Use the clay (ectoderm) in your kit. Roll the clay into a ball and then press the clay to make a thin flat oval (like a pancake). This represents the neural plate.

2. Over the next few days, a trench or dip forms in the neural plate. This creates a neural groove.
   - Make a trench or groove that runs down the middle of the neural plate—the neural groove.

3. By the 21st day of development, a neural tube begins to form when the edges of the neural groove meet in the middle to begin forming the spinal cord and brain.
   - Beginning in the middle of the neural groove, push the edges of the clay together to make a tube—the neural tube.

4. The closing of the neural tube is usually complete by the 28th day of pregnancy—before a woman is even aware that she is pregnant.
   - Continue to push the edges of the clay together to complete the closing of the tube—the neural tube.
The neural tube is the beginning of an embryo’s central nervous system. It grows and develops to form the brain and spinal cord. The anterior (front) part of the neural tube enlarges and develops into the brain. The posterior (rear) part of the neural tube develops into the spinal cord.

**Neural tube defects** may occur if the neural tube does not close properly. Spina bifida (an abnormal spinal cord) results when the posterior part of the neural tube fails to close. Anencephaly (an abnormal brain) results when the anterior part of the neural tube fails to close properly.

5. How could you change your model to illustrate a neural tube defect that would lead to spina bifida?

6. How could you change your model to illustrate a neural tube defect that would lead to anencephaly?

**IMPORTANT:** Return the “Ectoderm” (clay) to the bag and seal the bag.
Part 5: A Difficult Decision

During Anita’s fifth month of pregnancy, she has another sonogram. This sonogram reveals that her baby is a boy who has a severe type of spina bifida. With this type, a sac of fluid comes through an opening in the baby’s back. Part of the spinal cord and nerves are in this sac and are damaged. This type of spina bifida causes moderate to severe problems such as difficulty going to the bathroom, loss of feeling in the legs or feet, and lack of mobility in the legs.

Anita’s doctor explains that she needs to make a serious decision.

- She could have surgery performed on the baby immediately after it is born (postnatal surgery).

**OR**

- She could participate in a clinical trial (research study) that is being done to determine whether prenatal surgery (performed when the baby is still in the womb) for spina bifida is safer and more effective than postnatal surgery.

**Anita’s options**

<table>
<thead>
<tr>
<th>Participate in clinical trial</th>
<th>Do NOT participate in clinical trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scientific research needed to provide evidence that prenatal fetal surgery for spina bifida is safer and more effective than postnatal surgery.</td>
<td>ONLY allowed to receive postnatal surgery</td>
</tr>
</tbody>
</table>

Random assignment

- 50%  
- Experimental group: Receives prenatal fetal (before birth) surgery
- 50%  
- Control group: Receives postnatal (after birth) surgery
Anita really wants the prenatal surgery to close the hole in her baby’s spine while he is in the womb. She had heard wonderful things about the prenatal surgery. She feels that the prenatal surgery will give her baby the best chance for a normal life. Anita agreed to participate in the clinical trial because she feels that a 50/50 chance of having the prenatal surgery is better than no chance of having the prenatal surgery.

1. Why do half of the children in the clinical trial receive postnatal (after birth) surgery?

2. Flip a coin (there is a penny in your kit) to see whether Anita is assigned to the control group (HEADS) or the experimental group (TAILS). To what group is Anita assigned—the control group or the experimental group? ________________

3. Your lab kit contains two blue cards—Control Group and Experimental Group. ONLY open the blue card that corresponds with the results of your coin toss.
   - HEADS control group card
   - TAILS experimental group card

4. Read the information on the card. If you were Anita, how would you feel about being in this group?

Are you curious about what is on the other card? After you have answered question 4, you may open the other card if you wish.
5. Some patients, doctors, and scientists have expressed concerns about the ethical issues involved in the clinical trial that compared fetal surgery with postnatal surgery for spina bifida.

- Describe at least one reason why it was important to conduct this clinical trial.

- Describe at least one reason why people may feel that the design of this clinical trial raises ethical issues.

6. If you were a mother who was pregnant with a fetus who had a severe form of spina bifida, would you agree to participate in the clinical trial? Explain why or why not.
Part 6: Could neural tube defects be prevented?

Studies have shown that if all women who could become pregnant were to take a multivitamin with the B–vitamin folic acid, the risk of neural tube defects could be reduced by up to 70%. Folic acid is a water soluble B–vitamin that helps build healthy cells. Because it is water soluble, folic acid does not stay in the body for very long, so women need to take it every day to help reduce the risk of neural tube defects.

The U.S. Food and Drug Administration recommends that women of childbearing age (15–45) take 400 micrograms (mcg) of folic acid vitamin supplement daily, regardless whether they are planning a pregnancy or not. This is due to the fact that folic acid only works before women know they are pregnant. So if women wait to start folic acid until they know they are pregnant, it will likely be too late for the vitamin to offer protection from neural tube defects.

1. If Anita starts taking prenatal vitamins that contain folic acid after she knows she is pregnant, can this prevent neural tube defects? Explain your answer.

The U.S. Food and Drug Administration in 1996 authorized that all enriched cereal grain products be fortified with folic acid, with optional fortification beginning in March 1996 and mandatory fortification in January 1998. The data below shows the rates of spina bifida over the period of 1991 through 2005.
2. Based on the information in graph (on the previous page), *Spina Bifida Births in the United States, 1991–2005*, what conclusions can you draw about the impact of fortification of cereal grains with folic acid on the incidence of spina bifida?

Folic acid fortification reduces the incidence of spina bifida.

3. Based on the information in the table below, *Women’s Awareness of Folic Acid*, what conclusions can you draw about the understandings that women have about the importance of increasing their folic acid intake BEFORE becoming pregnant?

### Women’s Awareness of Folic Acid

<table>
<thead>
<tr>
<th>Women who:</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heard about folic acid in 1995</td>
<td>52%</td>
</tr>
<tr>
<td>Heard about folic acid in 1998</td>
<td>68%</td>
</tr>
<tr>
<td>Are not currently pregnant who take a multivitamin containing folic acid</td>
<td>29%</td>
</tr>
<tr>
<td>Know folic acid helps prevent birth defects</td>
<td>13%</td>
</tr>
<tr>
<td>Know folic acid should be taken before and during pregnancy</td>
<td>7%</td>
</tr>
</tbody>
</table>

4. State two actions that could be taken to increase the number of women who take folic acid supplements BEFORE they know they are pregnant?

Student answers will vary. Answers may include: public service announcements, information in health or biology classes, brochures given out at first visit to gynecologist, etc.
Part 7: Preventing other birth defects

There are many other types of birth defects. Some of these are inherited—caused by defective genes inherited from the parents. Others are a result of the environmental factors—exposure of an embryo or fetus to harmful substances or pathogens while they are in the uterus (womb). Others may be due to an interaction between genes and the environment. There are actions that a woman can take to reduce the risks of having a child with birth defects.

The exposure of an embryo or fetus to harmful substances or pathogens may have no effect, or they may cause major (obvious at birth) birth defects, or minor (subtle but noticed later in life) birth defects.

The chart below illustrates when developing organs may be affected by harmful substances or pathogens. The chart will help you think about the effects of the mother’s lifestyle on embryonic and fetal development.

- **Black bars** represent times when harmful substances may cause major birth defects such as missing limbs (arms or legs), cleft palate, or deformed brain or spinal cord structure.
- **Gray bars** represent times when harmful substances may cause minor birth defects such as low birth weight, slow mental development, deafness, or visual problems.

First sign of possible pregnancy—a late period

<table>
<thead>
<tr>
<th>Embryonic Stage (in weeks)</th>
<th>Fetal Stage (in weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ</td>
<td>1  2  3  4  5  6  7  8</td>
</tr>
<tr>
<td>Nervous System</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td></td>
</tr>
<tr>
<td>Arms &amp; Legs</td>
<td></td>
</tr>
<tr>
<td>Eyes</td>
<td></td>
</tr>
<tr>
<td>Teeth</td>
<td></td>
</tr>
<tr>
<td>Ears</td>
<td></td>
</tr>
</tbody>
</table>
1. An **embryo** is an unborn offspring in whom the major body organs are still forming. Once the major organs have formed, the unborn offspring is called a **fetus**. At the beginning of what week does an embryo become a fetus?

2. At which stage (the embryo stage or the fetus stage) is exposure to harmful substances, such as alcohol or drugs, most likely to cause **major** birth defects?

3. During which weeks might prenatal exposure to harmful substances lead to **major** birth defects that affect the nervous system?

4. During which weeks might prenatal exposure to harmful substances be most likely to cause **minor** structural or physiological (functional) defects that affect the nervous system?

5. Most women do not suspect they are pregnant until the end of week 3.
   - Why do doctors recommend that women who MAY become pregnant avoid X-rays, certain medications, drugs, alcohol, and other potentially harmful substances?
   - What parts of the developing baby may be harmed by exposure to harmful substances before the mother realizes she is pregnant?
Use the information in the Preventing Birth Defects brochure to answer the following questions.

6. Give four examples of chemicals or pathogens that can enter the fetus and cause birth defects.

- Alcohol
- Drugs
- Tobacco (smoking)
- Measles
- STD's
- etc.

7. Describe three examples of actions that could be taken during pregnancy to reduce the risks for birth defects caused by environmental factors.

- Do not take over-the-counter or prescription drugs unless recommended by a doctor.
- Take a vitamin pill.
- Live smoke-, alcohol- and drug-free.
- Have regular medical and dental check-ups.
- Get to a healthy weight before pregnancy and stay physically active.
- Avoid using hot tubs and saunas when pregnant or planning pregnancy.
- Eat a variety of foods.

8. Describe three examples of actions that could be taken before pregnancy to reduce the risks for birth defects caused by environmental factors.

Students should note that the same actions that are taken after a person knows they are pregnant should be taken before becoming pregnant.

- Do not take prescription drugs unless recommended by a doctor.
- Take a vitamin pill.
- Live smoke-, alcohol- and drug-free.
- Have regular medical and dental check-ups.
- Get to a healthy weight before pregnancy and stay physically active.
- Avoid using hot tubs and saunas when pregnant or planning pregnancy.
- Eat a variety of foods.
MATERIAL SAFETY DATA SHEET

1. PRODUCT AND COMPANY IDENTIFICATION

Product Name (as printed on the label): “AFP Indicator” (simulated)

Product identity: 0.1% bromothymol blue solution

Distributor: Wards Natural Sciences, 5100 West Henrietta Road. PO Box 92912, West Henrietta, NY 14692-9102

Telephone number for information: (800) 962-2660
Medical emergency phone number (Chemtrec): (800) 424-9300

Date of this MSDS: 5/16/13

2. COMPOSITION/INFORMATION ON INGREDIENTS

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>CAS Number</th>
<th>% Weight/Volume</th>
<th>TLV Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromothymol blue sodium salt</td>
<td>34722-90-2</td>
<td>0.1%</td>
<td>None established</td>
</tr>
<tr>
<td>Water</td>
<td>7732-18-5</td>
<td>99.9%</td>
<td>None established</td>
</tr>
</tbody>
</table>

3. HAZARDS IDENTIFICATION – for all pH buffer products

**EMERGENCY OVERVIEW**

Do not ingest. Avoid skin and eye contact. Avoid exposure to vapor or mists.

Potential Health Effects

- **EYES**: May cause irritation.
- **SKIN**: May cause slight irritation.
- **INHALATION**: n/a
- **INGESTION**: May cause gastrointestinal discomfort

4. FIRST AID MEASURES

- **EYES**: Flush with water for at least 15 minutes, raising and lowering eyelids occasionally. Get medical attention if irritation persists.
- **SKIN**: Thoroughly wash exposed area for at least 15 minutes. Remove contaminated clothing. Launder contaminated clothing before reuse. Get medical attention if irritation persists.
- **INGESTION**: Do not induce vomiting. If swallowed, if conscious, give plenty of water immediately and call a physician or poison control center. Never give anything by mouth to an unconscious person.

5. FIRE FIGHTING MEASURES

- **NFPA Rating**: Health: 1 (slight) Fire: 0 Reactivity: 0
- **Extinguisher Media**: Any means suitable for extinguishing surrounding fire
- **Firefighting Procedures**: Firefighters should wear full protective equipment and NIOSH approved self-contained breathing apparatus.
- **Unusual Fire and Explosion Hazards**: None

6. SPILL OR LEAK PROCEDURES

Ventilate area of spill. Clean-up personnel should wear proper protective equipment and clothing. Mop up, or absorb material with suitable absorbent and containerize for disposal.

7. HANDLING AND STORAGE

Store in a cool dry place. Handle using safe laboratory practices.
8. EXPOSURE CONTROLS/PERSOAL PROTECTION

Respiratory Protection: None required
Ventilation: Local Exhaust: Preferred
Protective Gloves: Natural rubber, Neoprene, PVC or equivalent.
Eye Protection: Splash proof chemical safety goggles should be worn.
Other Protective Clothing or Equipment: Lab coat, apron, eye wash, safety shower.

9. PHYSICAL AND CHEMICAL PROPERTIES

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting Point</td>
<td>&lt;2°C</td>
</tr>
<tr>
<td>Boiling Point</td>
<td>&gt;98°C</td>
</tr>
<tr>
<td>Vapor Pressure</td>
<td>Ca 50 @ 20°C</td>
</tr>
<tr>
<td>Vapor Density</td>
<td>~ same as water</td>
</tr>
<tr>
<td>Specific Gravity (H₂O=1)</td>
<td>~1</td>
</tr>
<tr>
<td>Percent Volatile by Volume</td>
<td>information not available</td>
</tr>
<tr>
<td>Evaporation Rate</td>
<td>~ same as water</td>
</tr>
<tr>
<td>Solubility in Water</td>
<td>soluble</td>
</tr>
<tr>
<td>Appearance and Odor</td>
<td>Green liquid</td>
</tr>
</tbody>
</table>

10. STABILITY AND REACTIVITY

Stability: Stable
Materials to Avoid: none known
Hazardous Decomposition Products: none
Reactive under what conditions: none known

11. TOXICOLOGICAL INFORMATION

<table>
<thead>
<tr>
<th>Toxicity (rat) LD₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute oral toxicity = information not available</td>
</tr>
<tr>
<td>Acute toxicity from vapor = information not available</td>
</tr>
</tbody>
</table>

Effects of Overexposure:
Acute: Irritation of eyes/skin
Chronic: Irritation of eyes/skin
Target Organs: Eyes, skin.
Primary Route(s) of Entry: Ingestion

12. ECOLOGICAL INFORMATION No data available

13. DISPOSAL CONSIDERATIONS

Waste Disposal Methods: Dispose in accordance with all applicable Federal, State and Local regulations. Always contact a permitted waste disposer (TSD) to assure compliance.

14. TRANSPORTATION INFORMATION No data available

15. REGULATORY INFORMATION No data available

16. ADDITIONAL INFORMATION

The information provided in this Material Safety Data Sheet represents data from the manufacturer and/or vendor and is accurate to the best of our knowledge. By providing this information, Science Take-Out LLC makes no guarantee or warranty, expressed or implied, concerning the safe use, storage, handling, precautions, and/or disposal of the products covered or the accuracy of the information contained in this fact sheet. It is the responsibility of the user to comply with local, state, and federal laws and regulations concerning the safe use, storage, handling, precautions, and/or disposal of products covered in this fact sheet.
MATERIAL SAFETY DATA SHEET

1. PRODUCT AND COMPANY IDENTIFICATION

<table>
<thead>
<tr>
<th>Label on Tube</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amniotic Fluid</td>
<td>Buffer pH 4</td>
</tr>
<tr>
<td>Anita's Blood Serum</td>
<td>Buffer pH 10</td>
</tr>
</tbody>
</table>

Distributor: Wards Natural Sciences, 5100 West Henrietta Road. PO Box 92912, West Henrietta, NY 14692-9102

Telephone number for information: (800) 962-2660
Medical emergency phone number (Chemtrec): (800) 424-9300

Date of this MSDS: 5/16/13

2. COMPOSITION/INFORMATION ON INGREDIENTS

<table>
<thead>
<tr>
<th>Product</th>
<th>Ingredients</th>
<th>CAS Numbers</th>
<th>% Weight/Volume (balance is water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 4 buffer</td>
<td>Potassium biphthalate</td>
<td>877-24-7</td>
<td>0.5%</td>
</tr>
<tr>
<td>pH 10 buffer</td>
<td>Sodium carbonate</td>
<td>497-19-8</td>
<td>0.25%</td>
</tr>
<tr>
<td></td>
<td>Sodium bicarbonate</td>
<td>144-55-8</td>
<td>0.15%</td>
</tr>
</tbody>
</table>

For all the ingredients
- NIOSH ILDH: none estab.

3. HAZARDS IDENTIFICATION – for all pH buffer products

**EMERGENCY OVERVIEW**

Do not ingest. Avoid skin and eye contact. Avoid exposure to vapor or mists.

**Potential Health Effects**
- EYES: May cause irritation.
- SKIN: May cause irritation.
- INHALATION: n/a
- INGESTION: May cause gastrointestinal discomfort and mouth burns.

4. FIRST AID MEASURES – for all pH buffer products

- EYES - Flush with water for at least 15 minutes, raising and lowering eyelids occasionally. Get medical attention if irritation persists.
- SKIN - Thoroughly wash exposed area for at least 15 minutes. Remove contaminated clothing. Launder contaminated clothing before reuse. Get medical attention if irritation persists.
- INGESTION - Do not induce vomiting. If swallowed, if conscious, give plenty of water immediately and call a physician or poison control center. Never give anything by mouth to an unconscious person.

5. FIRE FIGHTING MEASURES – for all pH buffer products

- NFPA Rating: Health: 1 Fire: 0 Reactivity: 0
- Extinguisher Media: Any means suitable for extinguishing surrounding fire
- Special Firefighting Procedures: Firefighters should wear full protective equipment and NIOSH approved self-contained breathing apparatus.
- Unusual Fire and Explosion Hazards: No data available

6. SPILL OR LEAK PROCEDURES – for all pH buffer products

Ventilate area of spill. Clean-up personnel should wear proper protective equipment and clothing. Absorb material with suitable absorbent and containerize for disposal.

7. HANDLING AND STORAGE – for all pH buffer products

Store in a cool dry place. This Material is not considered hazardous. Handle using safe laboratory practices.
8. EXPOSURE CONTROLS/PERSONAL PROTECTION – for all pH buffer products

   Respiratory Protection: n/a
   Ventilation: Local Exhaust: Preferred
               Mechanical(General): Acceptable
               Special: No
   Other: No

   Protective Gloves: Natural rubber, Neoprene, PVC or equivalent.
   Eye Protection: Splash proof chemical safety goggles should be worn.
   Other Protective Clothing or Equipment: Lab coat, apron, eye wash, safety shower.

9. PHYSICAL AND CHEMICAL PROPERTIES – for all pH buffer products

   Melting Point: ~0°C   Boiling Point: ~100°C
   Vapor Pressure: information not available   Vapor Density: information not available
   Specific Gravity (H₂O=1): ~1   Percent Volatile by Volume: >99
   Evaporation Rate: information not available   Solubility in Water: soluble
   Appearance and Odor: Clear colorless liquid

10. STABILITY AND REACTIVITY – for all pH buffer products

    Stability: Stable   Materials to Avoid: strong acids and bases
    Hazardous Decomposition Products: none known   Hazardous Polymerization: will not occur

11. TOXICOLOGICAL INFORMATION

    | Ingredient       | Toxicity (oral-rat) LD₅₀ |
    |------------------|--------------------------|
    | Potassium biphthalate | 3200 mg/kg              |
    | Sodium carbonate   | 4090 mg/kg              |
    | Sodium bicarbonate | 4220 mg/kg              |

    Effects of Overexposure (for all pH buffers):
    Acute: Essentially non-hazardous. Possible irritation of eyes/skin/stomach
    Chronic: None known.
    Conditions aggravated/Target organs: none known
    Target Organs: Eyes, skin, and gastrointestinal tract.
    Primary Route(s) of Entry: Ingestion or skin contact.

12. ECOLOGICAL INFORMATION – for all pH buffer products

    No ecological data available

13. DISPOSAL CONSIDERATIONS – for all pH buffer products

    Waste Disposal Methods: Dispose in accordance with all applicable Federal, State and Local regulations.
    Always contact a permitted waste disposer (TSD) to assure compliance.

14. TRANSPORTATION INFORMATION

    D.O.T. SHIPPING NAME: Not regulated

15. REGULATORY INFORMATION – for all pH buffer products

    EPA regulations: RCRA Hazardous waste number (40 CFR 261.33) – not listed
                     RCRS Hazardous waste classification (40 CFR 261) – not classified
                     SARA Toxic Chemical (40 CFR 372.65) – not listed
                     SARA EHS (Extremely Hazardous Substance (40 CFR 355) – not listed
    OSHA regulations: Air Contaminant (29 CFR 1910.1000) – not listed

16. ADDITIONAL INFORMATION

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