



The Biotechnology Education Company®



EDVO-Kit

910

**Isolation of Plant
Mitochondria & Chloroplasts**

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

In this experiment, students will learn to isolate and detect specific markers in mitochondria and chloroplasts from plant tissue.

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Isolation of Plant Mitochondria & Chloroplasts

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Each module is designed for 6 groups.

Storage:

Store components for Modules I & II in the freezer.

Experiment Components

Each experiment module is designed for 6 groups

A *Pisum sativum* (Pea seeds)

Module I: Isolation of Mitochondria

B1 Homogenization Buffer I
C1 Potassium Phosphate pH 7.4
D1 Assay Mix
E1 Ascorbic Acid
F1 Sodium Hydrosulfite

Module II: Isolation of Chloroplasts

G2 Homogenization Buffer II
H2 60% Sucrose in Tricine buffer pH 7.5
I2 Tricine buffer, 10x concentrate, pH 7.5

Also Included:

- 1 ml pipets
- 10 ml pipets
- Transfer pipets
- Microcentrifuge tubes
- Filters

All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

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Isolation of Plant Mitochondria & Chloroplasts

Requirements

- Vermiculite (horticulture grade)
- Nursery flat, 21 x 10 x 2 inches
- Scissors or razor blades
- Pasteur pipets
- Blender
- Funnels
- Cheesecloth
- Microscope, slides, and coverslips
- Centrifuge (refrigerated Sorvall SS-34, HB4 or equivalent)
- Clinical centrifuge (optional)
- Microcentrifuge
- Centrifuge tubes
- Small paint brushes
- Spectrophotometer (optional, must be capable of measuring 1 ml sample)
- Ice bucket and ice
- Waterbath
- Distilled water
- Isopropanol (100%)
- Ethanol (70% and 95%)
- Acetone (optional)
- Beakers or flasks
- Pipet pumps
- Automatic micropipet and tips
- Test tubes (13 x 100 mm)



Isolation of Plant Mitochondria & Chloroplasts

Biologists recognize that cells are the basic units of structure for all living things. The cells of prokaryotes-like bacteria are relatively homogeneous. Their cytoplasm is differentiated into regions that differ in appearance, but these regions are not physically distinct and are not subdivided by physical barriers like membranes. In eukaryotic cells, smaller membrane-bound structures are regularly found. These have recognizable names: nucleus, mitochondrion, chloroplast, vacuole, etc. and are called organelles. Organelles are physically distinct structures, suspended within the cytoplasm of the cell but separated from it. Organelles are sites of particular chemical reactions and are defined by their functions as well as by their structure. It is useful to ask, "How do we know what functions each of the organelles perform?"

Much of what we know about organelle function comes from investigations in which organelles were removed from the rest of the cell and studied in isolation. Techniques for isolating organelles from their cells are based on physical differences between them. In this experiment, chloroplasts and mitochondria will be isolated from plant cells using density gradient centrifugation.

Because organelles differ from one another in mass and density, they can be separated from one another by centrifugation. If any two particles are suspended in water, the larger or more dense of the two will sediment more quickly through the solution in response to gravity. When the particles are small as organelles, sedimentation can take a long time. In a centrifuge, the rate of sedimentation is increased. As the rotor of a centrifuge spins, centrifugal force (a force outward - away from the center of the rotation) is applied to the particles in solution. This force is much larger than the force of gravity. Because centrifugal force replaces gravitational force as the principal force acting on the solution, its strength is normally reported as "times the force of gravity" or "X g". In density gradient centrifugation, the solution in which the organelles are suspended is prepared in a centrifuge tube such that the solution is least dense at the top of the tube and most dense at the bottom. This is what is meant by "density gradient". In the centrifuge, organelles sediment through the solution until they reach a place in the tube where their own density matches that of the solution around them. For example, chloroplasts in cells are of the same density, when the centrifuge is stopped the chloroplasts are all found in the same location in the tube. They form a distinct green band in the gradient.

Using this technique, students will isolate two different organelles: chloroplasts and mitochondria. Students will calculate yield of chloroplasts by measuring chlorophyll and assay an enzyme present in mitochondria to determine the efficiency of their isolation procedures.

EXPERIMENT OBJECTIVE:

In this experiment, students will learn to isolate and detect specific markers in mitochondria and chloroplasts from plant tissue.

LABORATORY SAFETY

Standard laboratory precautions are required. Gloves and goggles should be worn in the laboratory.



CTAB, cetyltrimethylammonium bromide (in the extraction buffer) is a strong detergent and can cause burns to the skin. Follow your instructor's direction for proper handling and disposal of these materials.

MODULE 1A: Isolation of Mitochondria

All solutions should be kept ice-cold. Keep cell homogenate on ice while you are working.

1. Harvest 5 grams of 7-day-old pea roots, shake off vermiculite in which they are growing, and rinse in a beaker of distilled water.
2. Chop the roots into small pieces with a razor blade or scissors and put into a chilled blender with 20 ml ice-cold Homogenization Buffer I (diluted B1).

Note: More than one 5 gram batch of roots can be homogenized at a time. If several lab groups are sharing the blender, they should use 20 ml of buffer for each 5 grams of roots homogenized, then divide the homogenate.

3. Homogenize the tissue with five 2-3 second bursts of the blender at high speed.
4. Wearing gloves, filter the homogenate through four layers of cheesecloth plus one layer of Filters. It may be necessary to squeeze the filtrate through the cloth.
5. Pour the filtrate into a centrifuge tube, balance against a tube of water or against a tube from another lab group, and centrifuge at 4°C, 700 xg, 10 minutes (2500 rpm in a Sorvall SS34 rotor).
6. Decant the supernatant from step 5 into a clean centrifuge tube and centrifuge at 4°C, 10,000 xg, 10 minutes (9500 rpm in a Sorvall SS34 rotor).



MODULE 1A: Isolation of Mitochondria

- Decant the supernatant from step 6 into a beaker, label "10,000 xg supernatant" and store on ice. This will be used for the assay of the mitochondrial marker. The pellet at the bottom of the centrifuge tube should contain isolated mitochondria. Wash them by gently resuspending them in 20 ml of fresh homogenization buffer. This can be done by pipeting 1-2 ml of the buffer into the tube and using a small paint brush to break up the pellet. Once the pellet is resuspended in this small volume, it can be diluted with the remaining 18-19 ml of buffer.
- Re-centrifuge the washed mitochondria as in step 6 above.
- Discard the supernatant from this spin and resuspend the mitochondrial pellet in 5 ml of homogenization buffer. Label "mitochondria preparation" and store on ice.

MODULE 1B: Assay of Mitochondrial Marker Enzyme

Cytochrome c oxidase is a large complex of enzymes that catalyzes a whole series of reactions. The overall reaction catalyzed by the complex is:



2+ = reduced form of cytochrome c

3+ = oxidized form of cytochrome c

In this reaction, you are measuring how much color develops or changes over time. The more cytochrome c oxidase enzyme found in the mitochondria, the faster the color change. Cytochrome c is the substrate, ascorbic acid is a proton donor which allows the cytochrome c to be reduced so it can then be oxidized.

- From the ice bucket, remove 0.2 ml of "mitochondria preparation" to each of two small test tubes and 0.2 ml of "10,000 xg supernatant" to one small test tube and allow them to come to room temperature on the lab bench. To one of the tubes containing mitochondria, add 0.8 ml of Potassium Phosphate buffer (C1) for a 1:5 dilution (prep 1). To the other tube of mitochondria, add 1.8 ml of Potassium Phosphate buffer (C1) for a 1:10 dilution (prep 2). Store at 4°C.

Tube	mitochondria Preparation	Potassium Phosphate Buffer
Mito prep 1	0.2 ml	0.8 ml
Mito prep 2	0.2 ml	1.8 ml

MODULE 1B: Assay of Mitochondrial Marker Enzyme

- Assemble five reaction mixtures in five small test tubes. Label the tubes as follows: (a) blank, (b) reference, (c) 10,000 xg super., (d) Mitochondria prep 1 and (e) prep 2. Add 1 ml of distilled water to the blank tube and 0.8 ml 1x Assay Mix (diluted D1) to tubes b, c, d and e.
- Add 0.2 ml of Potassium Phosphate buffer (C1) to the reference tube (b) and 0.2 ml of each fraction to the appropriate tubes.

Experiment Procedure

Tube	Description	DH ₂ O	1x Assay Mix	10,000 xg Supernatant	Mito prep 1	Mito prep 2	Potassium Phosphate Buffer
a	blank	1 ml					
b	reference		0.8 ml				0.2 ml
c	10,000 xg super		0.8 ml	0.2 ml			
d	Mito prep 1		0.8 ml		0.2 ml		
e	Mito prep 2		0.8 ml			0.2 ml	

Note:

If your spectrophotometer uses a reference cuvette, this step is not necessary.

- Transfer the contents of the blank (tube A) to a cuvette and use it to blank the spectrophotometer. (Note: If your spectrophotometer uses a sample cuvette and a reference cuvette, both should be filled with distilled water to blank the instrument.) Place the reference sample into the spectrophotometer then measure and record the change in absorbance at 550 nm of this sample at 20 second intervals for 1 minute. This change represents non-enzymatic oxidation of the substrate. The rate of this change is used as a correction factor and should be subtracted from the rate of change when enzyme is present in step 5.

Note: If your spectrophotometer uses both sample and reference cuvettes, after blanking the instrument with water, place the reference solution into the reference cuvette and the enzyme-containing sample into the sample cuvette. Calculation of a correction factor as described above is not necessary.

- To perform the assay, transfer a reaction mix from each of the tubes containing sample (tubes c, d, and e) to a cuvette. Measure and record the absorbance for each sample at 550 nm at 20 second intervals for one minute to determine the rate of the reaction in the absence of substrate. This value (the slope of this line) will be used in step 7 below as a correction factor in your calculation of the rate of the enzyme-catalyzed reaction.
- Start the reaction (zero time) by adding 10 μ l of 0.8M ascorbic acid (dissolved E1) to the cuvette containing sample from tube c. Mix contents of the cuvette by inversion and quickly replace the cuvette into the spectrophotometer. Record the absorbance at 550 nm at 20 second intervals for 2 minutes.



MODULE 1B: Assay of Mitochondrial Marker Enzyme

7. Subsequently, repeat step 6 for samples d and e. You can scatter the readings as shown in the chart.

Molar concentration of enzyme in the preparation being assayed is not known. How is specific activity related to the rate of reaction just calculated? Specific activity = rate/mg protein (total) in the assay mixture.

Tube	0.8 M Ascorbic acid	Time	Reading
C (10,000 xg)	10 μ l	0	First reading
		20	
		40	
		60	
		80	
		100	
		120	2 min. last reading
D (Mito prep 1)	10 μ l	10	First reading
		30	
		50	
		70	
		90	
		110	
		130	2 min. last reading
E (Mito prep 2)	10 μ l	30	First reading
		50	
		70	
		90	
		110	
		130	
		150	2 min. last reading

MODULE 1B: Assay of Mitochondrial Marker Enzyme

8. Calculate the rate of Cytochrome c oxidation for each of the samples:

$$\text{Rate} = \frac{\text{Difference in Absorbance between steps 6 and 5/minute}}{\text{Molar absorptivity of Cytochrome c multiplied by path length of light}}$$

Molar absorptivity of cytochrome c = $18.5 \times 10^6 \text{ M}^{-1}$
 Path length through cuvette is usually 1 cm.

The units attached to rate are moles of Cytochrome c oxidized per minute. In general, this value would be reported as micromoles Cytochrome c oxidized per minute; thus, calculation is simplified to:

$$\text{Rate} = \frac{\text{Change in absorbance/minute (corrected as above)}}{18.5}$$

Note: Enzyme activity is usually reported in the literature as specific activity. This is a way of standardizing the reporting of enzyme activity since the exact



Module IIA: Isolation of Chloroplast

All solutions should be ice-cold. Keep solutions, samples, gradients, etc. on ice while you are working.

- Use 60% sucrose (H2) and 1x Tricine buffer (diluted I2) to prepare sucrose gradient as follows:

Solution Being Prepared	60% Sucrose	1x Tricine
50% sucrose	4.2 ml	0.8 ml
40% sucrose	6.7 ml	3.3 ml
30% sucrose	2.5 ml	2.5 ml

- Prepare sucrose gradient in a 50 ml centrifuge tube.
 - Pipette 5 ml 60% sucrose solution into bottom of the tube.
 - Layer 5 ml 50% sucrose, then 10 ml 40% sucrose into the tube. Layers should be distinct from one another if you are careful.

Hint: Tip the tube as you add each layer of sucrose. Let tip of the pipette just touch the surface of liquid in tube.
 - With the tip of a Pasteur pipette or stirring rod, gently mix at the interface of the 50% and 40% layers to diffuse slightly.
 - Layer 5 ml 30% sucrose on top of the gradient.
 - Keep the gradient on ice while you prepare tissue sample.
- Harvest 5 grams of 7-day-old pea seedlings at the soil line with a razor blade.
- Chop the tissue into small pieces with a razor blade or scissors and transfer them to a chilled blender containing 20 ml ice-cold homogenization buffer II (diluted G2).

Note: More than one 5 gram batch of seedlings can be blended at a time. If several lab groups are sharing the blender, they should use 20 ml of buffer for each 5 grams of seedlings homogenized, then divide the homogenate.
- Homogenize with five 2-3 second bursts of the blender at high speed.
- Filter the homogenate into a beaker (on ice) through four layers of cheesecloth, squeezing the cloth gently to remove most of the liquid (wear gloves).



Handle gradients very carefully, so as not to disturb the layers of sucrose.

Module IIA: Isolation of Chloroplast

Experiment Procedure



Use extreme caution when working with acetone. It is very flammable and should be handled in a fume exhaust hood.

7. Re-filter the first filtrate through one layer of Filter, moistened in homogenization buffer (G2), by gravity. Do not squeeze. You may want to prepare a wet-mount slide of the residue left in the cheesecloth or Filter for the microscope. What have you removed from the homogenate by filtration?
8. Layer 10 ml of the filtrate onto the top of the gradients (prepared in step 2). Balance your tube against the tube of another group for centrifugation. If necessary, add 1x homogenization buffer II to balance the tubes. Centrifuge at 4°C in HB4 rotor, 4000 rpm for 5 minutes, then increase speed to 10,000 rpm for 10 minutes. Allow the centrifuge to coast to a stop. Carefully remove your gradient from the rotor.
9. You should see two green bands in the gradient. The green band toward the bottom of the tube is the fraction containing intact chloroplasts. Remove the top of the gradient carefully with a Pasteur pipet. Save the two chlorophyll-containing fractions in clean tubes on ice.
10. Prepare wet-mount slides of the chlorophyll-containing fractions and examine on the microscope. What differences do you notice between them?

Read cautionary statement at left!

Module IIB: Quantitation of Chlorophyll in Chloroplasts (Optional Step)

1. Assay chlorophyll content of the two green bands. For each sample:
 - A. Into a clean centrifuge tube, pipette 50 µl of the gradient fraction to be assayed and 0.95 ml distilled water.
 - B. Add 4 ml acetone.
 - C. Centrifuge in a clinical centrifuge, 5 minutes.
 - D. Measure the absorbance of the solution in a spectrophotometer at 652 nm. (The appropriate blank for this measurement is 80% acetone in water.)
 - E. Calculate chlorophyll content:
 $A_{652} \times 29 = \mu\text{g chlorophyll}/10 \mu\text{l chloroplast fraction.}$



Study Questions

1. What fractions of marker enzyme activity are found in the 10,000 X g supernatant?
2. Explain the utility of marker enzymes in a cell fractionation experiment.
3. What are some reasons that marker enzyme activity might be formed in the "wrong" fraction?
4. What is Cytochrome c (the substrate for this marker enzyme) and what does it do in the mitochondrion?
5. Make a diagram of your chloroplast/sucrose gradient. Have materials other than chloroplasts banded in the tube? Where are they? What do they look like? Why was the cell homogenate filtered before being loaded on the gradient? What was removed by filtration? What fraction of the chloroplasts in the homogenate have you isolated intact?

Notes:

Experiment Procedure



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Instructor's Guide

Notes to the Instructor

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances.

If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK® web site. In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

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Pre-Lab Preparations**Tissue Requirement
Summary**

For Mitochondria:
5 g of freshly
harvested roots

For Chloroplasts:
5 g of freshly
harvested shoots

PLANT MATERIAL,**Component A *Pisum sativum* (pea seeds)
(for both modules)**

All of the experiments on cell fractionation included in this kit were planned using pea seedlings as starting material. From a single nursery flat (21 x 10 x 2 inches) enough plant material can be harvested to perform all three experiments, for each of the six lab groups. Plan to plant seeds one to two weeks before they will be used in the lab. To plant:

1. Soak the pea seeds (A) in a large beaker overnight.
2. Sow them in a layer of about 1.5 inches of wet horticulture grade vermiculite in a standard nursery flat. The seeds can be very close together.
3. Cover the seeds with 0.5 - 1.0 inch of vermiculite and water well.
4. Cover the flat with plastic wrap to hold in moisture while the seeds germinate.

Once the seedlings have begun to emerge from the vermiculite, remove the plastic and keep the flat well watered. Seedlings can be grown on a windowsill, in a growth chamber, or in the greenhouse. For best results, however, do not grow the seedlings under intense light. Under very bright lights, chloroplasts accumulate large starch granules which can disrupt the chloroplasts during isolation.

Module I: Use freshly harvested roots, 5 grams of tissue per lab group, for isolation of mitochondria.

Module II: For chloroplast isolation, use 5 grams of freshly harvested shoots per lab group. The shoots should be cut just at the soil line.

NOTE:

If Homogenization Buffer, component B1, has come out of solution, heat it to 65°C until it has completely dissolved.

Pre-Lab Preparations

PRE-LAB FOR MODULE I

1. Dilute Homogenization Buffer I (B1) by mixing 128 ml of component B1 with 192 ml of distilled water. Label and aliquot 50 ml per lab group and place on ice.
2. Cut cheesecloth to fit funnels. Arrange the filters and the cut cheesecloth so that they fit the funnels for filtering plant homogenates.
3. Aliquot 3 ml of 0.1 M Potassium Phosphate buffer (C1) for each group. Label 0.1 M Potassium Phosphate buffer.
4. Prepare reaction mixture for assay of mitochondrial marker enzyme. Add 1 or 2 granules of Sodium Hydrosulfite (F1) to the 10x Assay Mix (D1). Shake vigorously to dissolve the granules and mix the entire contents with 27 ml of distilled water. Label "1x Assay Mix" and aliquot 4 ml per group.
5. Dissolve Ascorbic Acid (E1) in 0.75 ml distilled water to make 0.8 M Ascorbic Acid. Label and aliquot 100 μ l per group.

PRE-LAB FOR MODULE II

1. Dilute Homogenization Buffer II (G2) by mixing 94 ml of component G2 with 47 ml distilled water. Label and aliquot 20 ml per lab group and place on ice.
2. Mix 5 ml of Tricine Buffer, 10x concentrate pH 7.5 (I2) with 45 ml of distilled water. Label and aliquot 8 ml per group.
3. Label and aliquot 20 ml of 60% Sucrose (H2) for each of the six groups.

For Module I, each student group requires:

- Ice and bucket
- 5 g 7-day-old pea roots
- Razor or scissors
- 50 ml ice cold diluted Homogenization Buffer I (diluted B1)
- Blender
- Cheesecloth
- Filters
- Funnel
- Centrifuge tubes (2 or 3)
- Small paint brush
- 3 ml 0.1 M Potassium Phosphate buffer (C1)
- 4 ml 1x Assay Mix (diluted D1)
- 100 μ l 0.8M Ascorbic Acid (dissolved E1)

For Module II, each student group requires:

- Ice and bucket
- 5 g 7-day-old pea shoots
- Razor or scissors
- 20 ml ice cold Homogenization Buffer II (diluted G2)
- 20 ml 60% sucrose solution (H2)
- 8 ml Tricine buffer (diluted I2)
- Blender
- Cheesecloth
- Filters
- Centrifuge tubes (2 or 3)
- 10 ml pipets and pumps
- Microscope slides and coverslips
- Microscope
- Spectrophotometer (optional)
- Acetone (optional)

**Please refer to the kit
insert for the Answers to
Study Questions**

 Material Safety Data Sheet May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.			
IDENTITY (As Used on Label and List) Homogenization Buffer I		Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.	
Section I Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, Zip Code) 1121 5th Street NW Washington DC 20001		Emergency Telephone Number 202-370-1500 Telephone Number for information 202-370-1500 Date Prepared 05-04-12 Signature of Preparer (optional)	
Section II - Hazardous Ingredients/Identify Information Hazardous Components [Specific Chemical Identity; Common Name(s)] OSHA PEL ACGIH TLV Other Limits Recommended % (Optional) CAS# 75277-39-3			
Section III - Physical/Chemical Characteristics			
Boiling Point	N/A	Specific Gravity (H ₂ O = 1)	N/A
Vapor Pressure (mm Hg.)	N/A	Melting Point	N/A
Vapor Density (AIR = 1)	N/A	Evaporation Rate (Butyl Acetate = 1)	N/A
Solubility in Water		Yes	
Appearance and Odor		Clear liquid	
Section IV - Physical/Chemical Characteristics			
Flash Point (Method Used)	Flammable Limits	LEL	UEL
Extinguishing Media Water spray, carbon dioxide, dry chemical powder			
Special Fire Fighting Procedures Wear SCBA and protective clothing to prevent contact with skin and eyes			
Unusual Fire and Explosion Hazards Emit toxic fumes under fire conditions			

Section V - Reactivity Data			
Stability		Unstable	Conditions to Avoid
		Stable	X
Incompatibility Strong oxidizing agents			
Hazardous Decomposition or Byproducts Carbon monoxide, carbon dioxide, nitrogen oxides, sulfur oxides			
Hazardous Polymerization		May Occur	Conditions to Avoid
		Will Not Occur	X
Section VI - Health Hazard Data			
Route(s) of Entry:		Inhalation?	Skin? Ingestion?
		Yes	Yes Yes
Health Hazards (Acute and Chronic) To the best of our knowledge, the chemical, physical and toxicological properties have not been thoroughly investigated			
Carcinogenicity:		NTP?	IARC Monographs? OSHA Regulation?
Signs and Symptoms of Exposure			
Medical Conditions Generally Aggravated by Exposure			
Emergency First Aid Procedures In case of contact, thoroughly flush eyes with copious amounts of water for at least 15 minutes			
Section VII - Precautions for Safe Handling and Use			
Steps to be Taken in case Material is Released for Spilled Sweep up, place in a bag and hold for waste disposal. Avoid raising dust. Ventilate area and wash spill site after material pick up is complete			
Waste Disposal Method Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner. Observe all federal, state, and local laws.			
Precautions to be Taken in Handling and Storing Store in a cool, dry place. Keep container closed.			
Other Precautions			
Section VIII - Control Measures			
Respiratory Protection (Specify Type) NIOSH/MSHA approved respirator			
Ventilation		Local Exhaust	Special
		Mechanical (General)	Other
Protective Gloves		safety gloves	Eye Protection safety goggles
Other Protective Clothing or Equipment		Safety shower and eye bath	
Work/Hygienic Practices Wash thoroughly after use			

 Material Safety Data Sheet May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.			
IDENTITY (As Used on Label and List) Homogenization Buffer II		Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.	
Section I Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, Zip Code) 1121 5th Street NW Washington DC 20001		Emergency Telephone Number 202-370-1500 Telephone Number for information 202-370-1500 Date Prepared 05-04-12 Signature of Preparer (optional)	
Section II - Hazardous Ingredients/Identify Information Hazardous Components [Specific Chemical Identity; Common Name(s)] OSHA PEL ACGIH TLV Other Limits Recommended % (Optional) CAS# 7447-40-7 Potassium Chloride Dextran T-40			
Section III - Physical/Chemical Characteristics			
Boiling Point	N/A	Specific Gravity (H ₂ O = 1)	N/A
Vapor Pressure (mm Hg.)	N/A	Melting Point	N/A
Vapor Density (AIR = 1)	N/A	Evaporation Rate (Butyl Acetate = 1)	N/A
Solubility in Water		Yes	
Appearance and Odor		Clear liquid, no odor	
Section IV - Physical/Chemical Characteristics			
Flash Point (Method Used)	Flammable Limits	LEL	UEL
Extinguishing Media Dry chemical, CO2, water spray or foam			
Special Fire Fighting Procedures Wear SCBA and protective clothing			
Unusual Fire and Explosion Hazards None known			

Section V - Reactivity Data			
Stability		Unstable	Conditions to Avoid
		Stable	X
Incompatibility BrF3			
Hazardous Decomposition or Byproducts N/A			
Hazardous Polymerization		May Occur	Conditions to Avoid
		Will Not Occur	X
Section VI - Health Hazard Data			
Route(s) of Entry:		Inhalation?	Skin? Ingestion?
		Yes	Yes Yes
Health Hazards (Acute and Chronic) No serious health effects in normal industrial uses. Dust may cause irritation of eyes, skin & mucous membs. Large doses by mouth may cause G.I disturb, vomiting & weakness			
Carcinogenicity:		NTP?	IARC Monographs? OSHA Regulation?
			N/A
Signs and Symptoms of Exposure Irritation of eyes, skin and mucous membranes, vomiting, weakness and circulatory problems			
Medical Conditions Generally Aggravated by Exposure			
Emergency First Aid Procedures Eyes and skin: Flush w/ copious amounts of water for 15 minutes. Inhalation: move to fresh air Ingestion: get medical attention. Never give anything by mouth to an unconscious person.			
Section VII - Precautions for Safe Handling and Use			
Steps to be Taken in case Material is Released for Spilled Sweep up material and place into suitable waste container. Flush spill area with water.			
Waste Disposal Method Dispose of material in accordance with state, local and federal regulations.			
Precautions to be Taken in Handling and Storing Store in tightly sealed containers. Protect from moisture.			
Other Precautions Avoid skin contact and avoid breathing dust.			
Section VIII - Control Measures			
Respiratory Protection (Specify Type) NIOSH approved dust respirator			
Ventilation		Local Exhaust	Special
		Mechanical (General)	Other
Protective Gloves		safety gloves	Eye Protection safety goggles
Other Protective Clothing or Equipment		Lab coat	
Work/Hygienic Practices Wash thoroughly after use			

 Material Safety Data Sheet May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.	
IDENTITY (As Used on Label and List) 10x Assay Mix	Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.
Section I	
Manufacturer's Name EDVOTEK, Inc.	Emergency Telephone Number 202-370-1500
Address (Number, Street, City, State, Zip Code) 1121 5th Street NW Washington DC 20001	Telephone Number for information 202-370-1500
	Date Prepared 05-04-12
	Signature of Preparer (optional)
Section II - Hazardous Ingredients/Identify Information	
Hazardous Components [Specific Chemical Identity; Common Name(s)] CAS# 9002-93-1 Potassium Phosphate Monobasic	OSHA PEL Triton X-100 Other Limits Recommended % (Optional)
Section III - Physical/Chemical Characteristics	
Boiling Point N/A	Specific Gravity (H ₂ O = 1) N/A
Vapor Pressure (mm Hg.) N/A	Melting Point N/A
Vapor Density (AIR = 1) N/A	Evaporation Rate (Butyl Acetate = 1) N/A
Solubility in Water Yes	
Appearance and Odor Reddish orange liquid	
Section IV - Physical/Chemical Characteristics N.D. = No data	
Flash Point (Method Used) No data	Flammable Limits LEL N.D. UEL N.D.
Extinguishing Media Water spray, carbon dioxide, dry chemical powder, alcohol or polymer foam	
Special Fire Fighting Procedures Wear SCBA and protective clothing to prevent contact with skin and eyes	
Unusual Fire and Explosion Hazards Emits toxic fumes under fire conditions	

Section V - Reactivity Data			
Stability	Unstable		Conditions to Avoid
	Stable	X	None
Incompatibility	Strong oxidizing agents, aluminum, steel, bases		
Hazardous Decomposition or Byproducts Carbon monoxide, carbon dioxide			
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None
Section VI - Health Hazard Data			
Route(s) of Entry:	Inhalation?	Yes	Skin? Yes Ingestion? Yes
Health Hazards (Acute and Chronic) May be harmful if inhaled or absorbed. Causes severe eye irritation			
Carcinogenicity: None identified NTP? IARC Monographs? OSHA Regulation?			
Signs and Symptoms of Exposure			
Medical Conditions Generally Aggravated by Exposure			
Emergency First Aid Procedures Immediately flush eyes/skin with copious amounts of water for at least 15 minutes. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen.			
Section VII - Precautions for Safe Handling and Use			
Steps to be Taken in case Material is Released for Spilled Cover with dry lime or soda ash, then pick up. Keep in closed container and hold for waste disposal. Ventilate area and wash spill site after material pickup is complete.			
Waste Disposal Method	Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.		
Precautions to be Taken in Handling and Storing Avoid contact and inhalation. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. Keep tightly closed. Store in a cool dry place.			
Other Precautions			
Section VIII - Control Measures			
Respiratory Protection (Specify Type) NIOSH/MSHA approved respirator			
Ventilation	Local Exhaust	Special	
	Mechanical (General)	Other	
Protective Gloves	Yes	Eye Protection	Safety goggles
Other Protective Clothing or Equipment Mechanical exhaust required			
Work/Hygienic Practices Wash thoroughly after handling			

 Material Safety Data Sheet May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.	
IDENTITY (As Used on Label and List) L-Ascorbic Acid Sodium	Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.
Section I	
Manufacturer's Name EDVOTEK, Inc.	Emergency Telephone Number 202-370-1500
Address (Number, Street, City, State, Zip Code) 1121 5th Street NW Washington DC 20001	Telephone Number for information 202-370-1500
	Date Prepared 05-04-12
	Signature of Preparer (optional)
Section II - Hazardous Ingredients/Identify Information	
Hazardous Components [Specific Chemical Identity; Common Name(s)] CAS# 134--03-2	OSHA PEL ACGIH TLV Other Limits Recommended % (Optional)
Section III - Physical/Chemical Characteristics	
Boiling Point 220°C	Specific Gravity (H ₂ O = 1) No data
Vapor Pressure (mm Hg.) No data	Melting Point No data
Vapor Density (AIR = 1) No data	Evaporation Rate (Butyl Acetate = 1) No data
Solubility in Water Yes	
Appearance and Odor White powder	
Section IV - Physical/Chemical Characteristics	
Flash Point (Method Used)	Flammable Limits LEL UEL
Extinguishing Media Water spray, carbon dioxide, dry chemical powder or appropriate foam	
Special Fire Fighting Procedures Wear SCBA and protective clothing to prevent contact with skin and eyes.	
Unusual Fire and Explosion Hazards Emits toxic fumes under fire conditions.	

Section V - Reactivity Data			
Stability	Unstable		Conditions to Avoid
	Stable	X	None
Incompatibility	N/A		
Hazardous Decomposition or Byproducts N/A			
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	
Section VI - Health Hazard Data			
Route(s) of Entry:	Inhalation?	Yes	Skin? Yes Ingestion? Yes
Health Hazards (Acute and Chronic)			
Carcinogenicity: NTP? IARC Monographs? N/A OSHA Regulation?			
Signs and Symptoms of Exposure Eyes: redness, tearing, blurred vision Skin: redness, itching, discomfort Inhal: coughing, chest discomfort, sneezing, sore throat Ingest: kidney/bladder stones, scurvy, nausea, diarrhea			
Medical Conditions Generally Aggravated by Exposure Interactions with certain medications may occur.			
Emergency First Aid Procedures Skin: wash with soap & copious amounts of water. Eyes: Flush with copious amounts of water. Inhal: remove to fresh air. Ingest: treat symptomatically and supportively.			
Section VII - Precautions for Safe Handling and Use			
Steps to be Taken in case Material is Released for Spilled Sweep up material and place into suitable waste container for disposal.			
Waste Disposal Method	Scoop up and place in a suitable container. Observe all local, federal and state regulations		
Precautions to be Taken in Handling and Storing Keep tightly closed and away from air, light, heat, sparks, open flames. Store in a cool, dry, well ventilated area away from incompatible substances.			
Other Precautions May burn but does not ignite readily. Avoid contact with strong oxidizers, excessive heat.			
Section VIII - Control Measures			
Respiratory Protection (Specify Type) NIOSH approved dust and mist respirator			
Ventilation	Local Exhaust	Special	
	Mechanical (General)	Other	
Protective Gloves	safety gloves	Eye Protection	safety goggles
Other Protective Clothing or Equipment lab coat			
Work/Hygienic Practices Wash thoroughly after use			

 Material Safety Data Sheet May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.			
IDENTITY (As Used on Label and List) Sodium Hydrosulfite		Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.	
Section I		Emergency Telephone Number 202-370-1500	
Manufacturer's Name EDVOTEK, Inc.		Telephone Number for information 202-370-1500	
Address (Number, Street, City, State, Zip Code) 1121 5th Street NW Washington DC 20001		Date Prepared 05-04-12	
		Signature of Preparer (optional)	
Section II - Hazardous Ingredients/Identify Information			
Hazardous Components [Specific Chemical Identity, Common Name(s)] OSHA PEL ACGIH TLV Other Limits Recommended % (Optional)			
CAS# 7775-14-6			
Section III - Physical/Chemical Characteristics			
Boiling Point	No data	Specific Gravity (H ₂ O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	>300°C
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data
Solubility in Water	Yes		
Appearance and Odor	White powder		
Section IV - Physical/Chemical Characteristics			
Flash Point (Method Used)	Flammable Limits	LEL	UEL
Extinguishing Media	Carbon dioxide, dry chemical powder, do not use water		
Special Fire Fighting Procedures	Wear SCBA and protective clothing to prevent contact with skin and eyes.		
Unusual Fire and Explosion Hazards	Emits toxic fumes under fire conditions. Reducing agent		

Section V - Reactivity Data			
Stability	Unstable		Conditions to Avoid
	Stable	X	Heat, sparks, and open flame
Incompatibility	Strong oxidizing agents, strong acids		
Hazardous Decomposition or Byproducts	Sulfur oxides		
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	
Section VI - Health Hazard Data			
Route(s) of Entry:	Inhalation?	Skin?	Ingestion?
	Yes	Yes	Yes
Health Hazards (Acute and Chronic)	Causes eye/skin irritation. Material is irritating to mucous membranes & upper respiratory		
Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
		N/A	
Signs and Symptoms of Exposure	Nausea, headache and vomiting		
Medical Conditions Generally Aggravated by Exposure			
Emergency First Aid Procedures	Immediately flush eyes/skin with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. Remove to fresh air. If not breathing give artificial respiration. Difficulty breathing-O ₂		
Section VII - Precautions for Safe Handling and Use			
Steps to be Taken in case Material is Released for Spilled Evacuate area. Shut off all sources of ignition. Wear SCBA, rubber boots and heavy rubber gloves Cover with dry lime, sand or soda ash. Place in covered containers using nonsparking tools - put outdoors			
Waste Disposal Method			
Precautions to be Taken in Handling and Storing Do not heat above 50°C			
Other Precautions			
Section VIII - Control Measures			
Respiratory Protection (Specify Type) NIOSH/MSHA approved respirator			
Ventilation	Local Exhaust	Special	
	Mechanical (General)	Other	
Protective Gloves	safety gloves	Eye Protection	safety goggles
Other Protective Clothing or Equipment	Safety shower and eye bath		
Work/Hygienic Practices	Wash thoroughly after handling. Do not breathe dust.		

 Material Safety Data Sheet May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.			
IDENTITY (As Used on Label and List) Tricine Buffer		Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.	
Section I		Emergency Telephone Number 202-370-1500	
Manufacturer's Name EDVOTEK, Inc.		Telephone Number for information 202-370-1500	
Address (Number, Street, City, State, Zip Code) 1121 5th Street NW Washington DC 20001		Date Prepared 05-04-12	
		Signature of Preparer (optional)	
Section II - Hazardous Ingredients/Identify Information			
Hazardous Components [Specific Chemical Identity, Common Name(s)] OSHA PEL ACGIH TLV Other Limits Recommended % (Optional)			
CAS # 5704-04-1			
Section III - Physical/Chemical Characteristics			
Boiling Point	No data	Specific Gravity (H ₂ O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data
Solubility in Water	Yes		
Appearance and Odor	Clear liquid		
Section IV - Physical/Chemical Characteristics			
Flash Point (Method Used)	Flammable Limits	LEL	UEL
Extinguishing Media	Water spray, carbon dioxide, dry chemical powder or appropriate foam		
Special Fire Fighting Procedures	Wear SCBA and protective clothing to prevent contact with skin and eyes.		
Unusual Fire and Explosion Hazards	Emits toxic fumes under fire conditions.		

Section V - Reactivity Data			
Stability	Unstable		Conditions to Avoid
	Stable	X	
Incompatibility	Strong oxidizing agents		
Hazardous Decomposition or Byproducts			
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	
Section VI - Health Hazard Data			
Route(s) of Entry:	Inhalation?	Skin?	Ingestion?
	Yes	Yes	Yes
Health Hazards (Acute and Chronic)	May cause eye/skin irritation. To the best of our knowledge, the chem, physical, and toxicological prop. have not been thoroughly invest.		
Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
		N/A	
Signs and Symptoms of Exposure			
Medical Conditions Generally Aggravated by Exposure			
Emergency First Aid Procedures	Immediately flush eyes and skin with copious amounts of water. Remove to fresh air, if not breathing give artificial respiration. If breathing is difficult, give oxygen.		
Section VII - Precautions for Safe Handling and Use			
Steps to be Taken in case Material is Released for Spilled Sweep up, place in a bag and hold for waste disposal.			
Waste Disposal Method Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber. Avoid raising dust. Ventilate area and wash spill site.			
Precautions to be Taken in Handling and Storing Chemical safety goggles, chem. resistant gloves, NIOSH/MSHA approved respirator, safety shower and eye bath, mechanical exhaust required			
Other Precautions Avoid contact with eyes, skin and clothing. Avoid prolonged or repeated exposure.			
Section VIII - Control Measures			
Respiratory Protection (Specify Type) NIOSH/MSHA approved respirator			
Ventilation	Local Exhaust	Special	
	Mechanical (General)	Other	
Protective Gloves	Chem. resistant gloves	Eye Protection	Chem. safety goggles
Other Protective Clothing or Equipment			
Work/Hygienic Practices			