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# Scaffold Bovine Dermis

## DEFINITION

Scaffold Bovine Dermis is a remodelable collagen scaffold derived from fetal or neonatal bovine skin. It is presented to the physician as a flat white sheet that is cut to size and hydrated in room-temperature sterile saline solution before implantation. The sterile material is surgically secured, onlayed, and/or packed into deficient soft tissues such as skin, tendon, muscle, and dura mater. In order to provide antimicrobial protection of the Scaffold Bovine Dermis, silver nitrate solution may be added to the Scaffold Bovine Dermis, resulting in ionic silver as a component. The source fetal or neonatal bovine skin is mechanically and chemically processed to isolate the dermis and remove cells and cellular components. To prevent the transmission of infectious disease, the manufacturing process has been validated to inactivate viruses potentially present in the source material. To prevent the spread of transmissible spongiform encephalopathies, the source material is acquired from appropriate geographic locations in accordance with relevant guidelines subject to governmental oversight. The product is inspected and tested to ensure that the product meets specifications.

## SPECIFIC TESTS

• **Histological Evaluation**

**Solutions**

**1% Acidified alcohol solution:** To 99 mL of 70% ethyl alcohol add 1 mL of hydrochloric acid (37.5%).

**Potassium alum solution:** Dissolve 100 g of potassium alum in 1000 mL of distilled water with the aid of heat and a magnetic stirrer.

**Hematoxylin–alcohol solution:** Dissolve 5 g of hematoxylin (see *Reagents, Indicators, and Solutions, Reagent Specifications*) in 50 mL of 100% ethyl alcohol at room temperature.

**Hematoxylin solution:** Slowly combine the 1000 mL of the *Potassium alum solution* with the 50 mL of the *Hematoxylin–alcohol solution*. Bring to a boil as rapidly as possible. Remove from heat, and slowly add 2.5 g of mercuric oxide. Return the solution to heat until it becomes dark purple, remove from heat, and cool in a sink of cold water.

**Eosin solution:** Dissolve 1.0 g of eosin Y, water soluble, in 100 mL of distilled water. Dissolve 1.0 g of phloxine B in 100.0 mL of distilled water. Combine 100 mL of eosin Y solution with 10 mL of phloxine B solution, 780 mL of 95% ethyl alcohol, and 4.0 mL of glacial acetic acid. [Note—Filter daily before use.]

**Bluing agent:** Dissolve 1.54 g of lithium carbonate in 100 mL of distilled water.

**10% Neutral buffered formalin:** To 6.5 g of dibasic sodium phosphate (anhydrous) and 4.0 g of monobasic sodium phosphate, add 900 mL of distilled water and 100 mL of formaldehyde (37%–40%).

**Sample preparation and staining:** Remove a sample of the finished product with an 8.0-mm biopsy punch. Place the sample in a labeled tissue cassette, and fix for 24 h in *10% Neutral buffered formalin*. Dehydrate the sample in sequential soaks of the following: 70% ethyl alcohol (45 min), 80% ethyl alcohol (45 min), 95% ethyl alcohol (90 min), 100% ethyl alcohol (180 min), and xylene (90 min). Embed the sample in melted paraffin, cool, and cut 5-µm-thick sections with a microtome. Collect sections on microscope slides. Deparaffinize the slides with xylene, and hydrate with distilled water. Stain in *Hematoxylin solution* for 6–15 min. Wash in running tap water for 2–5 min. Dip two times in *1% Acidified alcohol solution*. Wash briefly in tap water. Place in *Bluing agent* until the sections are bright blue. Wash in running tap water for 10 min. Place in 80% ethyl alcohol for 1–2 min. Dehydrate, and clear through two changes each of 95% ethyl alcohol, 100% ethyl alcohol, and xylene, 2 min each. Affix a coverslip over the tissue, using an appropriate resinous mounting medium. The nuclei stain blue, the cytoplasm stains from pink to red, and the collagen fibers stain from pink to red.

**Microscopic and morphological characteristics:** The collagen fibers of the Scaffold Bovine Dermis stain pink-red, and no evidence of cell nuclei or cytoplasm is apparent in prepared histological sections, as shown in the USP Bovine Acellular Dermal Matrix Reference Photomicrographs,[1] of products with acceptable histological appearance.

• **Protein Determination**

Use the Kjeldahl nitrogen (protein) determination method to calculate the percent protein of the final product, as directed in *Nitrogen Determination ⟨461⟩*, with the following specifics. Suitable equipment and procedures are readily available.[2]

**Digestion:** Prepare a rack of 15–20 Kjeldahl digestion tubes. In each, place 2.0–2.2 g of the final product, 0.2 ± 0.05 g of ammonium sulfate, a metallic catalyst tablet,[3] and boiling chips.[4] Prepare a blank tube with catalyst tablets and boiling chips (reagent blank). To each tube add

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15 mL of concentrated sulfuric acid, and then, very slowly, 3 mL of hydrogen peroxide (30%–35%). Place the digestion tubes on a digestion block, and heat to 410°. Digest for 60 ± 5 min. The mixture in the tubes should be a clear green.

**Distillation:** Add excess base (50% sodium hydroxide). Generally, for each 5 mL of concentrated sulfuric acid used in the digestion, 20 mL of 40% (w/w) sodium hydroxide is required to make the digest strongly alkaline (pH >11). Mix each tube, and let cool to room temperature. Distill each tube to collect approximately 125 mL of total distillate in a flask containing 25 mL of 4% boric acid. A reagent blank is run with each set.

**Titration:** Titrate the collected distillate with 0.2 N sulfuric acid to a neutral gray color endpoint. Record the volume of sulfuric acid used.

**Calculation:** Calculate the percentage of protein:

$$\text{Result} = [(\text{mL of sulfuric acid} - \text{mL of blank}) \times \text{N of sulfuric acid} \times A \times B]/\text{weight of sample (g)}$$

$A$ = milliequivalent weight N × 100 (%), 1.4007

$B$ = protein factor for meat, 6.25

**Acceptance criteria:** The percentage of protein in 2.0–2.2 g of the Scaffold Bovine Dermis sample is between 90.0% and 95.0%.

- **Lipid Analysis**

**Analysis:** A standard Soxhlet extraction apparatus is required. Dry flasks in an oven/desiccator and weigh, recording the weight to the nearest 0.0001 g. Grind or cut into small pieces 3.0–4.0 g of test material, and place into a thimble. Record the weight of the test material to the nearest 0.0001 g. Place the thimble of material and 80–90 mL of petroleum ether into an extraction flask, and place into the Soxhlet extraction tube. Reflux for 4 h. Collect all the ether into the flask, and evaporate. Weigh the flask, recording the weight to the nearest 0.0001 g. For the weight of lipid, subtract the weight of the clean flask from the final weight of the flask. Calculate the percentage of lipid on the basis of the weight of the starting material.

**Acceptance criteria:** The percentage of lipid in 3.0–4.0 g of the Scaffold Bovine Dermis sample is between 0% and 1.5%.

- **Loss on Drying ⟨731⟩:** Calculate the moisture content, with the following specifics. Mince approximately 5.0 g of Scaffold Bovine Dermis, and place it into an aluminum dish. Dry the sample in an air oven for 16–18 h at 100°–102°. Calculate the percentage of moisture in the sample taken:

$$\text{Dry matter \%} = [(\text{weight of dried sample + pan (g)} - \text{weight of pan (g)})/\text{g of sample}] \times 100$$

$$\text{Moisture \%} = 100 - \text{dry matter \%}$$

**Acceptance criteria:** The moisture loss is NLT 10.0% and NMT 12.0% of the original sample weight.

- **Ash Determination**

**Analysis:** Place a sample of the final product, about 5.0 g, in a kiln-dried porcelain crucible. Record the weight to the nearest 0.0001 g. Place the crucible containing the sample into an oven at 125° for 2–4 h. Then place the crucible containing the sample into a cool muffle furnace. Heat the furnace to 350°, and maintain the temperature until smoking ceases (generally about 20 min). Heat the furnace to 550°. Maintain the temperature for 2 h. Cool the crucible in a dessicator. Weigh the crucible, and record the weight to the nearest 0.0001 g. Calculate the percentage of ash:

$$\text{Result} = [(\text{weight of crucible + residue (g)} - \text{weight of crucible (g)})/\text{g of sample}] \times 100$$

**Acceptance criteria:** The percentage of ash is between 0% and 0.3%.

- **Carbohydrate Content**

Calculate the percentage of carbohydrates:

$$\text{Carbohydrate \%} = 100\% - (\text{protein \%} + \text{lipid \%} + \text{moisture \%} + \text{ash \%})$$

**Acceptance criteria:** The percentage of carbohydrates is equal to or less than 0.0%. Because this is a calculated value, influenced by the error inherent in the test methods above (*Protein Determination, Lipid Analysis, Loss on Drying,* and *Ash Determination*), a calculated value less than 0.0% is acceptable.

- **Gel Electrophoresis**

**Solutions**

**Collagen extraction solution:** Prepare a 0.5 M acetic acid solution containing 2 mM of ethylenediaminetetraacetic acid (EDTA).

**2X Tris-glycine sample buffer:** Prepare a 2X solution containing 63 mM Tris-HCl pH 6.8, 10% glycerol, 2% sodium dodecyl sulfate (SDS), 0.05% 2-mercaptoethanol, and 0.25% bromophenol blue.[5]

**1X Tris-glycine sample buffer:** Prepare a solution containing a mixture of *2X Tris-glycine sample buffer* and water (1:1).

**SDS-PAGE running buffer:** Prepare a solution containing 25 mM of Tris pH 8.3, 192 mM of glycine, and 0.1% of SDS.[6]

**Polyacrylamide gel:** Prepare a Tris-HCl polyacrylamide gel with a 4%–20% gradient.[7]

**Molecular weight marker:** Use a suitable molecular weight marker containing protein bands between 10 and 250 kilodaltons (kDa).

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**Staining solution:** Prepare a solution containing 0.25% (w/v) Coomassie brilliant blue R-250 (see *Reagents, Indicators, and Solutions, Reagent Specifications*) in 10% acetic acid and 10% *n*-propanol.

**Destain solution:** Prepare a mixture of water, acetic acid, and *n*-propanol (8:1:1).

**Collagen preparations:** Mince 0.5 g of Scaffold Bovine Dermis final product. Weigh a sample of minced Scaffold Bovine Dermis, and add to a volume of *Collagen extraction solution* to obtain a concentration of 5 mg/mL (dry weight of Scaffold Bovine Dermis). Extract on a rocking platform at room temperature for 72 h.

**Analysis:** Dilute acid-extracted collagen samples in *2X Tris-glycine sample buffer* to a concentration of 0.5 mg/mL, and incubate for 5 min at 100°. Load the *Polyacrylamide gel* in the electrophoresis apparatus, and add *SDS-PAGE running buffer* to the top and bottom reservoirs. Load 10 µL of *Molecular weight marker* in the first well of the *Polyacrylamide gel* and 10 µL of *1X Tris-glycine sample buffer* in the second well. Load 10 µL (5 µg) of each *Collagen preparation* into subsequent gel wells. Attach the cathode and anode to the appropriate terminals, and apply 110 V to the gel. Run the gel until the bromophenol blue reaches the bottom of the gel. Remove the gel from the electrophoresis apparatus, and place it in a tray containing enough *Staining solution* to cover the gel. Incubate the gel for 3 h on a rocker at room temperature. Completely remove the *Staining solution* from the tray, cover the gel with *Destain solution*, and slowly rock the gel for 20 min. Remove the *Destain solution*, and repeat the destaining procedure three times. Inspect the gel for bands that have migrated from the origin.

**System suitability:** All *Molecular weight marker* bands between 20 and 200 kDa are present. The lane containing *1X Tris-glycine sample buffer* does not contain any bands.

**Data analysis:** Where a protein band appears in the gel, the molecular weight of this protein is determined by comparing the position of the band to that of the known *Molecular weight marker*.

**Specificity and acceptance criteria:** The lanes of the *Polyacrylamide gel* that correspond to Scaffold Bovine Dermis show four major protein bands. Two bands, when compared to the *Molecular weight marker*, appear at 96 and 94 kDa. These two bands correspond to the monomeric alpha 1 and alpha 2 chains of collagen Type I, respectively. Another two bands appear close together at 200 kDa, which correspond to alpha 1 and alpha 1/alpha 2 collagen dimers.

- **Tensile Strength**

**Procedure:** Cut test specimens 5-mm wide × 50-mm long from representative pieces from final product lots. Measure the thickness of the specimens. Test the specimens with a commercially available material test system.[8] Mount and align each specimen, gripping 1 cm of the test specimen on both ends to ensure a test specimen gauge length of 3 cm. Pull the grips apart at 30 mm/min while concurrently measuring the force exerted on the specimen. Record the maximum force (*N*) measured during the test.

Calculate the tensile strength:

$$\text{Tensile strength } (N/\text{mm}^2) = \text{maximum force } (N)/5 \text{ (mm)} \times \text{thickness (mm)}$$

**Acceptance criteria:** The measured tensile strength for each lot is NLT 5 $N/\text{mm}^2$.

- **Suture Retention Force**

**Analysis:** Cut representative 1-cm × 1-cm test specimens from final product lots. Using an appropriate suture material (e.g., 4-0 polypropylene suture), thread the suture 3 mm from the edge of the sample in the center, and pull through. Clamp approximately 5 mm of the opposite, unsutured end of the test specimen in the upper pneumatic grip of a commercially available material test system.[9] The suture tails are hanging freely. Clamp the suture tails to the lower grip. Pull the grips apart at 20 mm/min while concurrently measuring the force exerted. Record the maximum force (*N*) measured.

**Acceptance criteria:** The suture retention force measured for each lot is NLT 5*N* for a 1-cm × 1-cm test sample of Scaffold Bovine Dermis.

- **Thermal Analysis**

**Analysis:** Heat a final product sample of approximately 10–20 mg at 2°/min from 30°–90°, hydrate with water, and measure the thermal characteristics of each processed sample with a differential scanning calorimeter as directed in *Thermal Analysis* ⟨891⟩.

**Acceptance criteria:** Scaffold Bovine Dermis displays a single thermal transition peak between 58° and 67°.

- **Visual Inspection**

**Analysis:** Visually inspect each piece of final product under a white light at a distance of 30–45 cm for color, the presence of particulates, and holes.

**Acceptance criteria:** Scaffold Bovine Dermis is white, and neither particulates nor holes are visible.

- **Hydration Rate**

**Analysis:** Cut a sample of finished product lot approximately 1 cm × 1 cm. Fully hydrate the sample, as indicated by a change in color from white to gray.

**Acceptance criteria:** The sample should be fully hydrated in NMT 3 min when placed in room-temperature saline solution.

- **Content of Silver**

**Sample:** Roll or fold a 4-cm × 4-cm sample of Scaffold Bovine Dermis into a labeled 50-mL Pyrex glass tube containing 30 mL of 6.0% nitric acid, and cap tightly. Heat the sample tube in a purified glycerin bath at 100°–105° for 16 h. Remove the sample tube from glycerin bath, and allow to cool to <30°. Transfer 0.5 mL of the dissolved *Sample* into a second Pyrex glass tube containing 30 mL of 2.0% nitric acid prepared freshly on the day of use, and cap tightly. The *Sample* should be stored at room temperature (15°–30°) until analysis is initiated.

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**Analysis:** The *Sample* is analyzed for ionic silver via inductively coupled plasma–atomic emission spectrometry (ICP–AES). The instrument should be a computer-controlled emission spectrometer with background correction capability. Accurately weigh the *Sample*. Nebulize the *Sample*, and transport the resulting aerosol to the plasma torch. Element-specific spectra are produced by radio frequency inductively conducted plasma. Spectra are dispersed by a grating spectrometer, and the intensities of the line spectra are monitored at specific wavelengths by a photosensitive device. Photo currents from the photosensitive device are processed and controlled by a computer system. A background correction technique is required to compensate for variable background contribution to the determination of silver. Background must be measured adjacent to the analyte wavelength during analysis. Four types of blanks are required for the analysis: The calibration blank is used in establishing the analytical curve. The laboratory reagent blank is used to assess possible contamination from the *Sample* preparation procedure. The laboratory fortified blank is used to assess routine laboratory performance. A rinse blank is used to flush the instrument uptake system and nebulizer between standards and to check solutions and samples to reduce memory interferences.

**Calibration and standardization:** Before using this method, the user must optimize the plasma operating conditions. The purpose of plasma optimization is to provide a maximum signal-to-background ratio. The use of a mass flow controller to regulate the nebulizer gas flow rate greatly facilitates the procedure.

**Data analysis and calculations:** Report results up to three significant figures as mg/kg on the dry weight basis. Calculate the concentration of silver in the *Sample*:

$$\text{Result} = [C \times V \times D]/W$$

$C$ = concentration in extract (mg/L)

$V$ = volume of extract (L)

$D$ = dilution factor (undiluted = 1)

$W$ = weight of sample aliquot extracted (g × 0.001 = kg)

**Acceptance criteria:** The content of ionic silver in the Scaffold Bovine Dermis must be more than 100.0 µg/cm$^2$ and less than 165.0 µg/cm$^2$ as determined by ICP–AES.

• **Sterility Tests ⟨71⟩:** It meets the requirements.

***Change to read:***

• **Bacterial Endotoxins Test ⟨85⟩:** It meets the requirements as directed in ▲*Medical Devices—Bacterial Endotoxin and Pyrogen Tests ⟨161⟩*.▲ (CN 1-May-2019)

**ADDITIONAL REQUIREMENTS**

• **Packaging and Storage:** The package is a sealed foil pouch that provides an effective moisture, light, gas, and sterility barrier. Store in clean, dry conditions between 15° and 30°.

• **Labeling:** Label it to indicate that it is derived from bovine origin. The product is labeled to indicate its intended clinical use. It is labeled with the dimensions of the product, the expiration date, the required storage conditions, the lot number, the part number, and the manufacturer's name and address. The label indicates that the product is sterile and nonpyrogenic and is designed for single-patient, one-time use. The labeling cautions the user to inspect the packaging for damage and to discard the product if the packaging has been compromised. The labeling also cautions the user to hydrate the product only in room-temperature sterile saline solution.

• **USP Reference Standards ⟨11⟩**

USP Authentic Visual References RS

USP Bovine Acellular Dermal Matrix Reference Photomicrographs.[1]

These photomicrographs show the histological appearance of failed, cell-containing source material (Photomicrographs 1 and 2) and of passing, processed, decellularized material (Photomicrographs 3 and 4). The samples were prepared as directed in the test for *Histological Evaluation* in *Specific Tests*.

[1] These photomicrographs are available as a CD from the USP Reference Standards collection, available to the reader through USP Customer Services. To order these and other Reference Standards, call 1-800-227-8772 (U.S. and Canada), +1-301-881-0666 or 00-800-4875-5555 (select Europe); or go online to www.usp.org. Order item number 1535824.

[2] A suitable device and associated procedures can be obtained from Labconoco, 8811 Prospect Ave., Kansas City, MO 64132-2696.

[3] A suitable catalyst is Pro-Pac CT-37, Alfie Packers, 8901 J St., Omaha, NE 68127.

[4] Commonly referred to as Henger granules.

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[5] A suitable sample buffer can be obtained from Invitrogen Corporation, 1600 Faraday Ave., P.O. Box 6482, Carlsbad, CA 92008.

[6] A suitable gel running buffer can be obtained from Bio-Rad Laboratories, 1000 Alfred Nobel Dr., Hercules, CA 94547.

[7] A suitable precast acrylamide gel can be obtained from Bio-Rad Laboratories, 1000 Alfred Nobel Dr., Hercules, CA 94547.

[8] A suitable material test system is available from Instron Corporation, 825 University Ave., Norwood, MA 02062-2643.

[9] A suitable material test system is available from Instron Corporation, 825 University Ave., Norwood, MA 02062-2643.

**Auxiliary Information** - Please check for your question in the FAQs before contacting USP.

| Topic/Question | Contact | Expert Committee |
|---|---|---|
| SCAFFOLD BOVINE DERMIS | Rebecca C. Potts<br>Associate Scientific Liaison | BIO52020 Biologics Monographs 5 - Advanced Therapies |
| REFERENCE STANDARD SUPPORT | RS Technical Services<br>RSTECH@usp.org | BIO52020 Biologics Monographs 5 - Advanced Therapies |

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