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Scaffold Porcine Bladder

DEFINITION

Scaffold Porcine Bladder is derived from porcine urinary bladder walls obtained from a certified closed pathogen-free herd. The bladder walls are processed by both mechanical and chemical methods that result in a sterile, decellularized material retaining a basement membrane with type IV collagen. Scaffold Porcine Bladder is manufactured in single and multi-layered lyophilized sheets as well as powder forms.

IDENTIFICATION

[Note—Powdered and multi-layered product forms must meet the requirements for *Identification* as single sheets before conversion to the other product types.]

- **A. Immunohistochemistry for Type IV Collagen**

Diluent: Tris-buffered saline (TBS) containing suitable amounts of normal serum, surfactant, and a preservative[1]

Primary antibody: Rabbit anti-human collagen IV,[2] cross-reactive with the corresponding porcine protein, diluted 1:500 in *Diluent*

Negative control solution: Diluted normal rabbit serum[3]

Secondary antibody: About 25 µg/mL of polymerized alkaline phosphatase-labeled anti-rabbit IgG[4] in *Diluent*

Sample preparations: Formalin-fixed, paraffin-embedded tissue samples are prepared, one per device, and sectioned at a thickness of 5 µm using suitable, validated methods. [Note—See also *Preparation of Biological Specimens for Histologic and Immunohistochemical Analysis* ⟨1285⟩ for helpful but not mandatory guidance.] Positive controls include normal porcine bladder, which is known to contain collagen IV at the location of the epithelial basement membrane. An additional sample of Scaffold Porcine Bladder is used as a negative control and incubated with *Negative control solution* rather than *Primary antibody* in the *Analysis*. One negative and one positive control section are used per test group. Before staining, sections are dewaxed with a suitable solvent[5] for 5 min, immersed in 100% ethanol for 5 min, then washed after the alcohol step and between each subsequent immunohistochemical step in a suitable buffer[6] three times for 5 min each. Note that all immersions and washes are done at ambient temperatures.

Analysis: *Sample preparations* are then treated for 10 min with a suitable enzyme[7] to retrieve the type IV collagen antigen followed by rinsing as above. Except for the negative control sample, the *Primary antibody* is then added for 30 min followed by rinsing as described above. For the negative control sample, *Negative control solution* is added to the sample for this incubation, rather than *Primary antibody*. Next the *Secondary antibody* is added for 30 min followed by rinsing as described above and then a final rinse in deionized water for 5 min. A suitable enzyme[8] substrate is added for 5 min followed by three rinses of 5 min each in deionized water to stop the reaction. The tissue is then stained with a hematoxylin solution[9] for 5 min followed by two rinses of 5 min each in deionized water then one rinse in a suitable buffer[10] for 5 min. The tissue is then placed in a clarifying buffer[11] for 10 min, followed by three washes for 5 min each in a suitable buffer. The tissue sections are then dehydrated in 100% alcohol, followed by xylene, and then a coverslip is mounted on each section using a suitable mounting medium.[12] Sections are examined with a light microscope at 40× magnification.

System suitability criteria: Negative controls should not contain any magenta staining, and positive controls should have magenta staining.

Acceptance criteria: Intense magenta staining (intensity of magenta staining should be similar to that observed with the positive controls) for the basement membrane protein type IV collagen must be observed on the luminal surface of the Scaffold Porcine Bladder sample.

- **B. Periodic Acid-Schiff Stain for Basement Membrane Structure**

Sample preparations: Formalin-fixed, paraffin-embedded tissue samples are prepared, one per device, and sectioned at a thickness of 5 µm using suitable, validated methods. [Note—See also ⟨1285⟩ for helpful but not mandatory guidance.] Positive controls include normal porcine bladder and a canine positive tissue control. Two positive control sections are used per test group. Before staining, sections are dewaxed with a suitable solvent[13] for 5 min, then immersed in 100% ethanol for 5 min, followed by a graded series of alcohols of 5 min each, ending in distilled water for 5 min.

Analysis: *Sample preparations* are placed in a 0.5% periodic acid solution[14] for 5 min followed by several washes in distilled water. The sections are then placed in a room temperature Schiff reagent solution[15] for 15 min, followed by rinsing with running, lukewarm water for

10 min. The sections are then immersed in a hematoxylin solution[16] for about 1 min, followed by rinsing in distilled water for 30 s. Sections are then placed in a bluing reagent[17] for 1 min, followed by rinsing in distilled water for 30 s. The stained sections are then placed in 95% ethanol for 1 min, then 100% ethanol for 1 min, then three washes in xylene for 1 min each, followed by mounting a coverslip over each section using a suitable mounting medium.[18] Stained sections are examined with a light microscope at 40× magnification.

System suitability criteria: Positive controls should have dark purple staining.

Acceptance criteria: Intense dark purple staining (intensity of dark purple staining should be similar to that observed with the positive controls) for basement membrane must be observed on the luminal surface of a single layer of scaffold.

SPECIFIC TESTS

• **Tensile Strength of Sheet Forms**

[Note—Powdered products must meet the requirement for *Tensile Strength of Sheet Forms* before conversion to powder.]

Sample preparation: Cut the test specimens using a dogbone die template with a narrow width of 0.125 in and an overall length of 2.50 in, from a representative of the final product.

Analysis: Hydrate the material. Test using an Instron material testing system. Set the distance between the grips to 1 in and the crosshead speed to 0.5 in/min (12.7 mm/min). Secure the test sample in the bottom and top test grips. Start the test and run until sample failure.

Calculations: To determine strain, take the extension at failure (l_f), subtract the extension at pre-load (l_i), and divide the difference by extension at pre-load (l_i). Multiply by 100 to convert to a percentage.

Acceptance criteria: >10% strain

• **Sterility Tests ⟨71⟩:** Meets the requirements

• **Bacterial Endotoxins Test ⟨85⟩:** It contains NMT 20 USP Endotoxin Units/device or per 1000 mg of powder.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Sheets are packaged in single-use, double barrier, peel-open pouches. Powder forms are stored in a glass vial or other container within a peel-open package. Store under clean, dry conditions, between 15° and 30°.

• **Labeling:** The package label indicates dimensions or mg, as applicable, of the Scaffold Porcine Bladder device, as well as the lot number, expiration date, and required storage conditions. The label is also marked “Sterile” followed by an “R” for irradiation method of sterilization.

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1 Included in Leica Biosystems kit catalog #AR9352 or a suitable alternative.

2 Abcam catalog #ab6586 or a suitable alternative.

3 Leica Biosystems catalog #PA0777 or a suitable alternative.

4 A suitable antibody source is catalog #BA-1000, from Vector, Burlingame, CA.

5 Leica Biosystems catalog #AR9222 or a suitable alternative.

6 Leica Biosystems catalog #AR9590 or a suitable alternative.

7 Leica Biosystems catalog #AR9551 or a suitable alternative.

8 Included in Leica Biosystems kit catalog #DS9390 or a suitable alternative.

9 Dako catalog #CS700 or a suitable alternative.

10 Leica Biosystems catalog #AR9590 or a suitable alternative.

11 Leica Biosystems catalog #3802918 or a suitable alternative.

12 ThermoScientific catalog #8312-4 or a suitable alternative.

13 Leica Biosystems catalog #AR9222 or a suitable alternative.

14 Richard Allen catalog #87007 or a suitable alternative.

15 Richard Allen catalog #87007 or a suitable alternative.

16 Richard Allen catalog #87007 or a suitable alternative.

[17] Richard Allen catalog #7301 or a suitable alternative.

[18] ThermoScientific catalog #8312-4 or a suitable alternative.

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

Topic/Question	Contact	Expert Committee
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