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Tissue Human Amnion Chorion Membrane Dehydrated

DEFINITION

Tissue Human Amnion Chorion Membrane Dehydrated is a dried bi-layer allograft containing amnion and chorion, derived from donated human placental tissues obtained from scheduled Caesarian sections. Using sterile solutions and aseptic techniques, the amniotic membrane is dissected from the placenta, the amnion and chorion layers are separated and then washed, layered, and dried. The product is provided in a sterile sheet form. Placentas used to produce Tissue Human Amnion Chorion Membrane Dehydrated are obtained from donors that have passed all applicable donor eligibility requirements and relevant communicable disease testing.

SPECIFIC TESTS

- **Histological Evaluation**

Hydration solution: 0.9% (w/v) sodium chloride (NaCl)

Cryopreservation matrix: Optimum cutting temperature (OCT) formulation of water-soluble glycols and resins[1]

Tissue preparation: Cut a sample of Tissue Human Amnion Chorion Membrane Dehydrated NLT 0.5 cm × 0.5 cm and NMT 1.0 cm × 1.0 cm. Hydrate the sample in 5 mL of *Hydration solution* for 10–30 min. Add a thin layer of the *Cryopreservation matrix* into the bottom of a suitable embedding mold,[2] and freeze on dry ice for 5–10 min until the *Cryopreservation matrix* appears white. Add more *Cryopreservation matrix* on top of the frozen layer, and place the tissue sample into the *Cryopreservation matrix*. Use forceps to arrange the tissue in the *Cryopreservation matrix* in the desired orientation for sectioning. Place the tissue sample and embedding mold onto dry ice for 10–20 min until the *Cryopreservation matrix* is completely solid and appears white. Immediately place the frozen block in a −80° freezer until ready for sectioning.

Cryosectioning: Add a layer of liquid *Cryopreservation matrix* onto the cryostat sample holder of a suitable cryostat,[3] and then place the frozen tissue block onto the holder in the desired orientation for sectioning. Freeze the tissue block onto the holder in the cryostat sectioning chamber before trimming the frozen block. Using the cryostat, trim the frozen block as necessary in 50–100 µm sections until the tissue sample is accessed. Subsequently, cut 5-µm section(s) of the tissue sample. Press each section onto the positively charged side of a microscope slide,[4] allowing the section to freeze onto the slide. Store the sections at −80° until use.

Staining preparation: When ready to stain, thaw the frozen slides at room temperature for NLT 30 min. Fix the tissue slides in ice cold acetone for 10 min. Allow the slides to dry at room temperature for NLT 30 min.

- **Verhoeff Staining**

Verhoeff staining solution: Prepare fresh Verhoeff stain containing 60 mL of 5% alcoholic hematoxylin, 24 mL of 10% aqueous ferric chloride, and 24 mL of Lugol's iodine working solution.[5]

Analysis: Place the slides in 100 mL of deionized water twice for 5 min each. Submerge the slides in *Verhoeff staining solution* for 15 min. Place the slides in five changes of deionized water until the water appears clear, then perform two additional changes in 100 mL of deionized water for 5 min each. Dip the slides in 100 mL of 2% aqueous ferric chloride 10 times, and then allow the stain to differentiate for 60 s until the amnion begins to appear gray. Place the slides in two changes of 100 mL of deionized water for 5 min each. Submerge the slides in 100 mL of 5% aqueous sodium thiosulfate for 1 min, then submerge the slides in two exchanges of 100 mL of deionized water for 5 min each.

Add 3–4 drops of a mounting solution[6] to each slide and incubate for 5 min. Carefully place a coverslip over the tissue section, taking care not to trap any bubbles under the coverslip. Seal the edges of the coverslip with clear nail polish, and store the slides at 4° protected from light.

Image the tissue sections using a suitable microscope at 40× magnification with brightfield.

Acceptance criteria: The product must show the presence of both the amnion layer and the chorion layer. The amnion layer contains a thin, dark staining layer followed by light staining throughout the rest of the amnion. The chorion layer must show a thin layer of light staining followed by dark staining throughout the rest of the chorion.

- **Human Type IV Collagen Immunostaining**

Phosphate buffered saline (PBS): 0.01 M phosphate buffer, 0.0027 M potassium chloride (KCl), and 0.137 M sodium chloride (NaCl), pH 7.4

1% Bovine serum albumin (BSA): 500 mg of BSA in 50 mL of *PBS*

Blocking buffer diluent: Add 50 µL of *t*-octylphenoxypolyethoxyethanol to 48.95 mL of *1% BSA*.

Blocking buffer: 2% (v/v) normal goat serum in *Blocking buffer diluent*

Human type IV collagen antibody solution: Dilute a suitable mouse anti-human type IV collagen antibody[7] (1:100) in *1% BSA*.

Secondary antibody solution: Dilute a suitable fluorescent labeled, goat anti-mouse antibody[8] 1:100 in *PBS*.

Sample: A sample of Tissue Human Amnion Chorion Membrane Dehydrated cut to NLT 0.5 cm × 0.5 cm and NMT 1.0 cm × 1.0 cm and prepared as directed in *Histological Evaluation* (see *Tissue preparation, Cryosectioning,* and *Staining preparation*).

Analysis: Place the slides in two exchanges of 100 mL of *PBS* for 5 min each. Circle each tissue section with a hydrophobic pen to make a hydrophobic ring surrounding the *Sample*. Place the slides into a humidity chamber for the following incubation steps. Add 50–100 µL of *Blocking buffer* to completely cover each tissue section within the hydrophobic ring, and incubate for 20 min. Blot off the excess *Blocking buffer*, but do not rinse the slides. Add 50–100 µL of *Human type IV collagen antibody solution* to completely cover each tissue section and incubate overnight for 12–24 h at 4°.

In parallel, prepare a negative control by incubating a *Sample* with 50–100 µL of *1% BSA* alone without primary antibody for the same amount of time followed by the same steps described below. Blot off the primary antibody or *1% BSA*, and rinse the slides by immersing in two exchanges of 100 mL of *PBS* for 5 min each. Add 50–100 µL of *Secondary antibody solution* to completely cover each tissue section, and incubate for 30 min. Blot off the *Secondary antibody solution* and place the slides in two exchanges of 100 mL of *PBS* for 5 min each, followed by 100 mL of deionized water for 5 min.

Add 3–4 drops of a mounting solution[9] to each slide and incubate for 5 min. Carefully place a coverslip over the tissue on each microscope slide, taking care not to trap any bubbles under the coverslip. Seal the edges of the coverslip with clear nail polish, and store the slides at 4° protected from light.

Image the tissue sections using a suitable fluorescent microscope at 40× magnification with an excitation wavelength of 628 nm and an emission wavelength of 692 nm. Other optical filters can also be used to image tissue autofluorescence relative to that seen with the specific fluorescence of the collagen type IV stain.

Acceptance criteria: Tissue Human Amnion Chorion Membrane Dehydrated must show positive staining for human type IV collagen in the amnion layer and throughout the chorion layer.

• **Ball-Burst Testing**

Normal saline: 0.9% (w/v) sodium chloride (NaCl)

Sample: Tissue Human Amnion Chorion Membrane Dehydrated with dimensions of NLT 25 mm × 25 mm. [Note—At least five *Samples* should be tested for each lot.] Hydrate the *Samples* in *Normal saline* for NLT 30 min. [Note—Complete the *Analysis* rapidly so the *Sample* does not dry before testing.]

Analysis: Center the *Sample* between the ball-burst specimen clamp plates. Ensure that the test article is flat over the hole of the ball-burst fixture. Trim the *Sample* if it extends beyond the fixation screws so that the *Sample* rests flat between the screws. Tighten the screws on the ball-burst fixture on either side of the *Sample* to secure the *Sample* in place. Place the ball-burst attachment in the hole of the ball-burst clamp, with the ball of the attachment resting on the *Sample*. The polished steel ball is 6.35 mm in diameter, and the diameter of the test area is 10.7 mm. Place the ball-burst specimen clamp with the *Sample* and the ball-burst attachment approximately centered on the lower compression plate of a suitable materials testing system.[10] Test the *Sample* by lowering the upper compression piston until the *Sample* fails, at a rate of 305 ± 13 mm/min. Record the load, in Newtons (N), at failure.

Acceptance criteria: NLT 0.25 N per *Sample*

• **Water Determination ⟨921⟩**

Sample: Weigh a sample of Tissue Human Amnion Chorion Membrane Dehydrated. The test sample should be NLT 40 and NMT 100 mg. [Note—Multiple grafts may be used when needed to create a sample size of 40 mg.]

Acceptance criteria: NMT 15%

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

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Tissue Human Amnion Chorion Membrane Dehydrated is packaged and supplied sterile in a double pouch configuration. Tissue Human Amnion Chorion Membrane Dehydrated can be stored between 0° and 38°.

• **Labeling:** The label shows the tissue identification number, expiration date, and size of Tissue Human Amnion Chorion Membrane Dehydrated contained in the pouch.

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[1] A suitable cryopreservation embedding medium can be obtained from Fisher Scientific, product #23-730-571 or a suitable equivalent.

[2] A suitable embedding mold is Thermo Scientific Shandon, product #22–19 or an equivalent.

[3] A suitable cryostat can be obtained from Thermo Scientific, product Microm HM 525 or a suitable equivalent.

[4] A suitable microscope slide can be obtained from Fisherbrand, product #12-550-15; Superfrost or a suitable equivalent.

[5] A suitable reagent can be obtained from Poly Scientific, product #K059-16OZ or a suitable equivalent.

[6] A suitable reagent can be obtained from Electron Microscopy Sciences/Diatome, product #17985-10 or a suitable equivalent.

[7] A suitable antibody can be obtained from Abcam, product #ab6311 Mouse Monoclonal Antibody to Human Type IV Collagen or a suitable equivalent.

[8] A suitable reagent can be obtained from Life Technologies, product #A-21054 or a suitable equivalent.

[9] A suitable reagent can be obtained from Electron Microscopy Sciences/Diatome, product #17985-10 or a suitable equivalent.

[10] A suitable materials system is the INSTRON Series 5500 Load Frames with a Series 2525 Load Cell or a suitable equivalent.

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

Topic/Question	Contact	Expert Committee
TISSUE HUMAN AMNION CHORION MEMBRANE DEHYDRATED	Rebecca C. Potts Associate Scientific Liaison	BIO32020 Biologics Monographs 3 - Complex Biologics and Vaccines
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	BIO32020 Biologics Monographs 3 - Complex Biologics and Vaccines

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