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Add the following:

^Scaffold Human Amniotic Membrane Allograft

DEFINITION

Scaffold Human Amniotic Membrane Allograft is derived from normal human placental amniotic tissue obtained from full-term pregnancies. The amniotic tissue is isolated and decellularized to remove cellular contaminants, resulting in an extracellular matrix with basement membrane. Scaffold Human Amniotic Membrane Allograft is composed predominantly of collagen and elastin. Tissues are obtained from sources that have passed applicable donor tissue eligibility requirements for relevant diseases, are processed, dehydrated, and terminally sterilized with e-beam irradiation. Scaffold Human Amniotic Membrane Allograft is supplied in a dry sheet form and is stored at ambient temperature.

IMPURITIES

• ANALYSIS OF RESIDUAL SODIUM DEOXYCHOLATE

Solution A: Purified water liquid chromatography–mass spectrometry (LC/MS) grade

Solution B: Acetonitrile LC/MS grade

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
8	25	75
9	2	98
13	95	5

Diluent 1: Acetonitrile and purified water, both LC/MS grade (50:50), and 0.1% formic acid

Diluent 2: Isopropyl alcohol and purified water (90:10)

Diluent 3: 50% ethanol in phosphate buffered saline (PBS)

Lock mass stock solution: Add 2.5 mL of purified LC/MS grade water to a 10-mg vial of leucine enkephalin acetate to obtain a 4-µg/µL stock solution.

Lock mass solution: 2 ng/µL prepared as follows. Transfer a 10-µL aliquot of the *Lock mass stock solution* to a 20-mL volumetric flask, and add *Diluent 1* to volume.

Calibration solution: 5 mM sodium formate in *Diluent 2*

Sodium deoxycholate stock solution 1: Weigh a 25 ± 0.5-mg portion of sodium deoxycholate, add to a 50-mL volumetric flask, and dissolve in *Diluent 3* to obtain a 500-µg/mL solution.

Sodium deoxycholate stock solution 2: Dilute *Sodium deoxycholate stock solution 1* volumetrically at a ratio of 1:50 in *Diluent 3* to obtain a 10-µg/mL solution.

Sodium deoxycholate standard solution: Dilute *Sodium deoxycholate stock solution 2* volumetrically at a ratio of 1:5 in *Diluent 3* to obtain a 2-µg/mL solution.

Lithocholic acid stock solution 1: Weigh a 25 ± 0.5-mg portion of lithocholic acid, add to a 50-mL volumetric flask, and dissolve in *Diluent 3* using a sonicator for approximately 30 min to obtain an approximate 500-µg/mL stock solution.

Lithocholic acid stock solution 2: Dilute *Lithocholic acid stock solution 1* volumetrically at a ratio of 1:50 in *Diluent 3* to obtain an approximate 10-µg/mL solution.

Lithocholic acid standard solution: Dilute *Lithocholic acid stock solution 2* volumetrically at a ratio of 1:5 in *Diluent 3* to obtain a 2-µg/mL solution.

Spiked internal standard solution 1: Dilute *Sodium deoxycholate standard solution* and *Lithocholic acid standard solution* in *Diluent 3* at a ratio of 1:1:18.

Spiked internal standard solution 2: Dilute *Sodium deoxycholate stock solution 2* and *Lithocholic acid stock solution 2* in *Diluent 3* at a ratio of 1:1:3.

Sample: 2 mg of Scaffold Human Amniotic Membrane Allograft. Subdivide the test and control sample(s) to facilitate transfer into 20-mL vials.

Spiked sample: Spike the sample with 60 µL of *Lithocholic acid standard solution*. Dry under a stream of nitrogen for 2 h.

Spiked control sample: Spike the sample with 60 µL of *Spiked internal standard solution 2*. Dry under a stream of nitrogen for 2 h.

Chromatographic system

Mode: UPLC

Detector: UV 190–400 nm

Column: 50-mm × 2.1-mm; 1.7-µm packing L7

Column temperature: 35°

Flow rate: 0.3 mL/min

Injection volume: 5 µL

Run time: 15 min

Mass spectrometry instrumental conditions

Mass analyzer: Quadrupole time of flight (QToF)

Mode of MS ionization: Electrospray ionization, negative ion mode

MS mode: Sensitivity

Spectral range: 50–1500 *m/z*

Detector: Time of flight (ToF)

Lock mass: 554.26 Da

Capillary: 2.6 kV

Sampling cone: 30 V

Extraction cone: 4 V

Temperatures

Source: 140°

Desolvation: 450°

System suitability

Sample: *Spiked internal standard solution 1*

Suitability requirements

Percent relative standard deviation (% RSD) detection limit: NMT 15%

Quality control check: 70%–130% of theoretical concentration

Relative response factor: 0.5–1.3

Deoxycholate–molecular mass (M–H): 391.2848 ± 0.02 Da

Internal standard–molecular mass (M–H): 375.2899 ± 0.02 Da

Analysis

Samples: *Spiked sample* and *Spiked control sample*

Sample extraction: Add *Diluent 3* to each sample vial containing *Spiked sample* or *Spiked control sample* using a ratio of 1.2 mL of *Diluent 3* per 2 mg of the sample. For each vial, extract the sample at 37 ± 2° for 120 ± 12 min. After the extraction is complete, remove the sample from each extraction vessel using a clean inert article, such as a disposable glass Pasteur pipette. Store the extract at 2°–8°.

Sample analysis: Analyze the extracts using instrument conditions described. Analyze 6 replicates of *Spiked internal standard solution 1* and *Diluent 3* as solvent blank as appropriate. Perform quality control (QC) checks at least every 10 samples using *Spiked internal standard solution 1*.

Analysis with mass spectrometer: Change the QToF mass spectrometer settings as follows.

Capillary: 2.6 kV

Sampling cone: 30.0 V

Extraction cone: 4 V

Temperatures

Source: 140°

Desolvation: 450°

Desolvation gas flow: 750 L/min

Use the *Calibration solution* to calibrate the mass spectrometer for a mass range of 50–1500 Da in sensitivity mode using tune page settings and without collision energy. Set up lock mass using *Lock mass solution* with a capillary voltage of 2.6 kV in negative mode of ionization at 554.26 Da (M–H).

Calculate the ratio of the deoxycholate peak area to the lithocholic acid peak area from the *Spiked sample* and the *Spiked control sample*.

Acceptance criteria: The ratio of the deoxycholate peak area to the lithocholic acid peak area from the *Spiked sample* must be less than that from the *Spiked control sample*.

• RESIDUAL CELL ANALYSIS

Harris hematoxylin stock solution: Dissolve hematoxylin¹ in alcohol and 20 mL of water. Add ammonium aluminum sulfate in water with heat until completely dissolved. Remove the ammonium aluminum sulfate solution from heat and mix with the hematoxylin solution. Bring the mixed solution to a rapid boil. Immediately remove from heat, add sodium iodate, and mix. Reheat the solution to boiling and boil for approximately 2–3 min. Remove from heat and plunge the solution container into an ice bath. Allow the solution to cool and pass through a grade 541 or comparable filter paper. Store the solution in a light-protected bottle at room temperature. This solution is stable for approximately 2 months.

Harris hematoxylin working solution: Add 4 mL of glacial acetic acid to 100 mL of filtered *Harris hematoxylin stock solution*.

Eosin Y stock solution (1%): Add 1.0 g of eosin Y to 100 mL of distilled water and mix well. [NOTE—This solution can be stored for up to 1 year at room temperature.]

Phloxine B stock solution: Add 1.0 g of phloxine B to 100 mL of distilled water and mix well. [NOTE—This solution can be stored for up to 1 year at room temperature.]

Eosin–phloxine B working solution: Add 100 mL of *Eosin Y stock solution (1%)* and 10 mL of *Phloxine B stock solution* to 780 mL of 95% alcohol, then add 4 mL of glacial acetic acid and mix well. [NOTE—This solution can be stored for up to 1 year at room temperature.]

Samples: 5-mm × 5-mm pieces of Scaffold Human Amniotic Membrane Allograft prepared as follows. Place each piece of trimmed tissue into a pre-labeled tissue cassette. Place the cassettes into a tissue processor cassette basket that is set into a container of 10% formalin.

Tissue processing: Tissue processing can be done using an automated tissue processor programmed using a stepwise schedule with all steps done under a vacuum, except the first 10% neutral formalin step. The stepwise procedure is provided in [Table 2](#).

Table 2

Step Solution	Time	Retort Temperature
10% neutral formalin	10 min	ambient
70% alcohol	15 min	ambient
80% alcohol	15 min	ambient
95% alcohol	15 min	ambient
95% alcohol	20 min	ambient
Absolute alcohol	15 min	ambient
Absolute alcohol	15 min	ambient
Absolute alcohol	20 min	ambient
Xylene	15 min	ambient
Xylene	20 min	ambient
Paraffin	20 min	61°
Paraffin	20 min	61°
Paraffin	20 min	61°