Histological characterization—

Buffered formalin and *Preparation of tissue for staining*—Proceed as directed in the test for *Histological characterization* under *Cryopreserved Human Fibroblast-Derived Dermal Substitute,* substituting Human Fibroblast-Derived Temporary Skin Substitute for Cryopreserved Human Fibroblast-Derived Dermal Substitute. The fibroblasts appear elongated and spindle shaped. The tissue contains about 10^6 cells per cm^2 and about 500 cells per mm along the section.

HEMATOXYLIN–EOSIN STAINING—

Hematoxylin–alcohol solution, Hematoxylin staining solution, 10% Acid alcohol, Eosin solution, and *Procedure*—Proceed as directed for *Hematoxylin–eosin staining* in the test for *Histological characterization* under *Cryopreserved Human Fibroblast-Derived Dermal Substitute.* Using *USP Human Fibroblast-Derived Skin Substitute Reference Photomicrograph 1* (hematoxylin–eosin stained) for comparison, the nylon-scaffold mesh, silicone membrane, and secreted collagen-based matrix are present and the tissue contains normal human fibroblast distributed throughout the secreted matrix and resembles normal human papillary dermis.

COLLAGEN STAINING—

Bouins's solution, Weigert's iron hematoxylin working solution, Gomori's trichrome solution, 1% Acetic acid, and *Procedure*—Proceed as directed for *Collagen staining* in the test for *Histological characterization* under *Cryopreserved Human Fibroblast-Derived Dermal Substitute.* Using *USP Human Fibroblast-Derived Skin Reference Photomicrograph 2* for comparison, collagen is found throughout the extracellular matrix in a manner indistinguishable from Cryopreserved Human Fibroblast-Derived Dermal Substitute.

DISTRIBUTION OF FIBRONECTIN—

Tris-saline buffer, 3% Hydrogen peroxide, Diaminobenzidine solution, Hematoxylin staining solution, and *Procedure*—Proceed as directed for *Distribution of fibronectin* in the test for *Histological characterization* under *Cryopreserved Human Fibroblast-Derived Dermal Substitute.* Using *USP Human Fibroblast-Derived Skin Substitute Reference Photomicrograph 3* (diaminobenzidine–hematoxylin stained) for comparison, fibronectin binds to collagen and is found throughout the extracellular matrix in a manner indistinguishable from Cryopreserved Human Fibroblast-Derived Dermal Substitute.

Metabolic activity assessment—

DPBS working solution, Assay stock medium, MTT-assay solution, MTT formazan calibration solutions, and *Procedure*—Proceed as directed in the test for *Metabolic activity assessment* under *Cryopreserved Human Fibroblast-Derived Dermal Substitute,* substituting Human Fibroblast-Derived Temporary Skin Substitute for Cryopreserved Human Fibroblast-Derived Dermal Substitute: the absorbance value of individual Human Fibroblast-Derived Temporary Skin Substitute sections at 540 nm is less than 0.1.

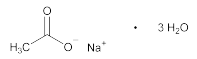
DNA content—

Cell culture water, Working DNA extraction buffer, Dilution buffer, DPBS without Ca^{++}, Mg^{++} solution, Calf thymus DNA calibration solutions, DNA staining solution, and *Procedure*—Proceed as directed in the test for *DNA content* under *Cryopreserved Human Fibroblast-Derived Dermal Substitute,* substituting Human Fibroblast-Derived Temporary Skin Substitute for Cryopreserved Human Fibroblast-Derived Dermal Substitute. The amount of DNA in individual Human Fibroblast-Derived Temporary Skin Substitute 11- × 11-mm sections is between 6 and 14 µg.

Total collagen content—

DPBS without Ca^{++}, Mg^{++} solution, DPBS with Ca^{++}, Mg^{++} solution, Collagenase extraction solution, 2% Acetic acid solution, Collagen calibration standards, Sirius red solution, 1% (p-tert-Octylphenoxy)polyethoxyethanol solution, and *Procedure*—Proceed as directed in the test for *Total collagen content* under *Cryopreserved Human Fibroblast-Derived Dermal Substitute,* substituting Human Fibroblast-Derived Temporary Skin Substitute for Cryopreserved Human Fibroblast-Derived Dermal Substitute: the amount of collagen in individual Human Fibroblast-Derived Temporary Skin Substitute 11- × 11-mm samples is between 0.50 and 4.0 mg.

Sodium Acetate



$C_2H_3NaO_2 \cdot 3H_2O$ 136.08

$C_2H_3NaO_2$ 82.03

Acetic acid, sodium salt, trihydrate;
Sodium acetate trihydrate [6131-90-4].
Anhydrous [127-09-3].

DEFINITION

Sodium Acetate contains three molecules of water of hydration, or is anhydrous. It contains NLT 99.0% and NMT 101.0% of $C_2H_3NaO_2$, calculated on the dried basis.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨**191**⟩**:** Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL,** *Acetate* ⟨**191**⟩**:** Meets the requirements

  Sample solution (for Lanthanum Nitrate test)**:** 10 mg/mL of sodium acetate in water. Adjust with 0.1 N sodium hydroxide to a slightly alkaline pH.

ASSAY

- **PROCEDURE**

  Sample solution: Equivalent to 200 mg of anhydrous sodium acetate in 25 mL of glacial acetic acid. [NOTE—Warm gently and mix to dissolve, if necessary.]

  Analysis: Add 2 drops of *p*-naphtholbenzein TS to the *Sample solution*, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 8.203 mg of $C_2H_3NaO_2$.

  Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

Inorganic Impurities

- **CHLORIDE AND SULFATE,** *Chloride* ⟨**221**⟩**:** A portion equivalent to 1.0 g of anhydrous sodium acetate shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (350 ppm).
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨**221**⟩**:** A portion equivalent to 10 g of anhydrous sodium acetate shows no more sulfate than corresponds to 0.50 mL of 0.020 N sulfuric acid (50 ppm).
- **CALCIUM AND MAGNESIUM**

  Sample solution: Equivalent to 10 mg/mL of anhydrous sodium acetate

  Analysis: To 20 mL of *Sample solution* add 2 mL each of 6 N ammonium hydroxide, ammonium oxalate TS, and dibasic sodium phosphate TS.

  Acceptance criteria: No turbidity is produced within 5 min.
- **POTASSIUM**

  Sample solution: Equivalent to 600 mg/mL of anhydrous sodium acetate

  Analysis: To 5 mL of *Sample solution* add 1 N acetic acid dropwise until the solution is slightly acidic, and then add 5 drops of sodium cobaltinitrite TS.

  Acceptance criteria: No precipitate is formed.
- **ALUMINUM** ⟨**206**⟩**:** Where it is labeled as intended for use in hemodialysis, proceed as directed in the chapter, using a portion equivalent to 10 g of sodium acetate trihydrate to prepare the *Sample solution.*

  Acceptance criteria: 0.2 ppm
- **HEAVY METALS,** *Method I* ⟨**231**⟩

  Standard solution: 1 mL of *Standard Lead Solution* and 11 mL of water in a 50-mL color-comparison tube

  Sample stock solution: Dissolve the equivalent of 4.2 g of anhydrous sodium acetate in water to make 50 mL

Sample solution: 12 mL of *Sample stock solution* in a 50-mL color-comparison tube
Monitor solution: 11 mL of *Sample stock solution* and 1.0 mL of *Standard Lead Solution* in a 50-mL color-comparison tube
Analysis: Proceed as directed in the chapter, omitting the dilution to 50 mL.
Acceptance criteria: NMT 10 ppm

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 7.5–9.2, in a solution equivalent to 30 mg/mL of anhydrous sodium acetate in carbon dioxide-free water
- **Loss on Drying** ⟨731⟩**:** Dry a sample at 120° to constant weight: the hydrous form loses 38.0%–41.0% of its weight, and the anhydrous form loses NMT 1.0% of its weight.
- **Insoluble Matter**
  Sample: Equivalent to 20 g of anhydrous sodium acetate
  Analysis: Dissolve the *Sample* in 150 mL of water, heat to boiling, and digest in a covered beaker on a steam bath for 1 h. Filter through a tared filtering crucible, wash thoroughly, and dry at 105°.
  Acceptance criteria: The weight of the residue is NMT 10 mg (0.05%).

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Label it to indicate whether it is the trihydrate or is anhydrous. Where Sodium Acetate is intended for use in hemodialysis, it is so labeled.

Sodium Acetate Injection

» Sodium Acetate Injection is a sterile solution of Sodium Acetate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of CH_3COONa.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.

Labeling—The label states the sodium acetate content in terms of weight and of milliequivalents in a given volume. Label the Injection to indicate that it is to be diluted to appropriate strength with water or other suitable fluid prior to administration. The label states also the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

USP Reference standards ⟨11⟩**—**
USP Endotoxin RS

Identification—It responds to the tests for *Sodium* ⟨191⟩ and for *Acetate* ⟨191⟩.

Bacterial endotoxins ⟨85⟩**—**It contains not more than 3.90 USP Endotoxin Units per mEq.

pH ⟨791⟩: between 6.0 and 7.0.

Particulate matter ⟨788⟩: meets the requirements under *Small-volume Injections.*

Other requirements—It meets the requirements under *Injections* ⟨1⟩.

Assay—

Standard stock solution—Dissolve 570.0 mg of sodium chloride, previously dried at 105° for 2 hours, in 100 mL of water, transfer to a 1000-mL volumetric flask, dilute with water to volume, and mix. Each mL of this solution contains 224 µg of sodium, equivalent to 800 µg of anhydrous sodium acetate.

Standard preparations—Transfer to each of four 100-mL volumetric flasks 10 mL of a nonionic wetting agent (1 in 500). Dilute the contents of one of the flasks with water to volume to provide a blank. To the remaining flasks add, respectively, 5.0, 10.0, and 15.0 mL of *Standard stock solution*, dilute with water to volume, and mix.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 800 mg of anhydrous sodium acetate, to a 1000-mL volumetric flask, dilute with water to volume, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask containing 10 mL of a nonionic wetting agent (1 in 500), dilute with water to volume, and mix.

Standard graph—Set a flame photometer for maximum transmittance at a wavelength of about 589 nm. Adjust the instrument to zero transmittance with the blank, and to 100% transmittance with the most concentrated of the *Standard preparations.* Read the transmittances of the other *Standard preparations*, and plot transmittances versus equivalent concentration of sodium acetate.

Procedure—Adjust the instrument as directed under *Standard graph*, read the transmittance of the *Assay preparation*, and calculate the sodium acetate content, in mg per mL, of Injection.

Sodium Acetate Solution

» Sodium Acetate Solution is an aqueous solution of Sodium Acetate. It contains not less than 97.0 percent and not more than 103.0 percent (w/w) of the labeled amount of $C_2H_3NaO_2$.

Packaging and storage—Preserve in tight containers.

Identification—It responds to the tests for *Sodium* ⟨191⟩ and for *Acetate* ⟨191⟩.

pH ⟨791⟩: between 7.5 and 9.2, when diluted with carbon dioxide-free water to contain 5% of solids.

Insoluble matter—Dilute a quantity of Solution, equivalent to 20 g of anhydrous sodium acetate, with water to 150 mL, heat to boiling, and digest in a covered beaker on a steam bath for 1 hour. Filter through a tared filtering crucible, wash thoroughly, and dry at 105°: the weight of the residue does not exceed 1 mg (0.005%).

Chloride ⟨221⟩**—**A quantity of Solution, equivalent to 1.0 g of anhydrous sodium acetate, shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.035%).

Sulfate ⟨221⟩**—**A quantity of Solution, equivalent to 10 g of anhydrous sodium acetate, shows no more sulfate than corresponds to 0.50 mL of 0.020 N sulfuric acid (0.005%).

Calcium and magnesium—Dilute a quantity of Solution, equivalent to 1.0 g of anhydrous sodium acetate, to 100 mL with water. To 20 mL of this solution add 2 mL each of 6 N ammonium hydroxide, ammonium oxalate TS, and sodium phosphate TS: no turbidity is produced within 5 minutes.

Potassium—To a quantity of Solution, equivalent to 3.0 g of anhydrous sodium acetate, add 0.2 mL of sodium bitartrate TS: no turbidity is produced within 5 minutes.

Heavy metals, *Method I* ⟨231⟩**—**Dilute a quantity of Solution, equivalent to 2.0 g of anhydrous sodium acetate, with water to 25 mL, and use glacial acetic acid instead of 1 N acetic acid for adjustment of the pH: the limit is 0.001%.

Assay—Weigh accurately about 1 g of Solution into a 250-mL conical flask, cautiously add (in a fume hood) 2.6 mL of acetic anhydride, mix, and allow to stand for 5 minutes. Add 25 mL of glacial acetic acid and 2 drops of *p*-naphtholbenzein TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, using 0.5 mL of water, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 8.203 mg of $C_2H_3NaO_2$.