This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

Designation: D7818 – 12 (Reapproved 2021)

Standard Test Method for Enumeration of Proteolytic Bacteria in Fresh (Uncured) Hides and Skins[1]

This standard is issued under the fixed designation D7818; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers the enumeration of bacteria that can hydrolyze protein/collagen in fresh (uncured) hides and skins. This test method is applicable to uncured hides and skins.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.4 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D6715 Practice for Sampling and Preparation of Fresh or Salt-Preserved (Cured) Hides and Skins for Chemical and Physical Tests

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

3. Summary of Test Method

3.1 Samples of uncured hides and skins are serially diluted and plated on agar containing casein from skim milk. The plates are incubated under aerobic conditions at 35 °C for 48 h. After incubation, to determine bacteria that can hydrolyze protein (proteolytic), the plates are flooded with dilute acid and the colonies showing a halo are counted.

4. Significance and Use

4.1 This test method enumerates proteolytic bacteria. Proteolytic bacteria have been known to cause damage to hides and skins.

5. Apparatus

5.1 *Incubator,* 35 ± 1 °C.

5.2 *Colony counter*—(not mandatory, but highly recommended).

5.3 *Sterile pipets.*

5.4 *Bent glass rods,* sterile.

5.5 *Stomacher,* for mixing initial dilution. (If stomacher is unavailable, hand-mix.)

5.6 *Balance.*

5.7 *Sterile petri dishes.*

5.8 *Autoclave (sterilizer).* (Check the effectiveness of sterilization weekly. For example, place spore suspensions or strips of *Bacillus stearothermophilus* (commercially available) inside glassware for a full autoclave cycle. Follow manufacturer's directions for sterilization of specific media.)

5.9 *Stomacher bags,* or sterile, sealable quart plastic bag (for example, food storage type, sterile bag).

5.10 *Cutting tool,* sterile (for example, scalpel blade and forcep, as needed for cutting fresh hides and skins).

5.11 *Vortex mixer,* for mixing dilution tubes (optional).

5.12 *pH meter.*

5.13 *Waterbath,* 45 ± 1 °C.

5.14 *Autoclave thermometer.*

6. Reagents and Materials

6.1 *5 % acetic acid.*

6.2 *Butterfield's Phosphate Stock Solution:* Dissolve 34 g KH_2PO_4 (Potassium Phosphate monobasic) in 500 mL DI

[1] This test method is under the jurisdiction of ASTM Committee D31 on Leather and is the direct responsibility of Subcommittee D31.02 on Wet Blue.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

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