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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 18654.13-2026

Replacing GB/T 18654.13-2008

Inspection of germplasm for fishes - Part 13: Test method for isozyme electrophoresis

鱼类种质检验 第13部分：同工酶电泳分析

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Standardization Administration of the PRC

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Foreword

This document complies with the provisions of GB/T 1.1-2020 "Standardization Work Guidelines Part

1.Structure and Drafting Rules of Standardization Documents". Drafting. This document is Part 13 of GB/T 18654, "Fish Germplasm Inspection". GB/T 18654 has already published the following parts.

1.Inspection Rules;

2.Sampling Methods;

3.Characteristic Determination;

4.Measurement of Age and Growth;

5.Dietary Analysis;

6.Determination of reproductive performance;

7.Ecological Characteristics Analysis;

8.Determination of Oxygen Consumption Rate and Critical Asphyxia Point;

9.Meat Percentage Determination;

10.Determination of muscle nutrient composition;

12.Karyotype Analysis;

13.Isozyme Electrophoresis Analysis;

1 Scope

GB/T 18654.13-2026 is the Chinese national standard covering isozyme electrophoresis - the older genetic marker method, still used because it is cheap and because decades of reference data for Chinese fish stocks exist in this form. It replaces GB/T 18654.13-2008 and has been in force since 1 September 2026, one of the parts of the GB/T 18654 fish germplasm inspection series revised together. It was issued on 27 February 2026 and has been in force since 1 September 2026, replacing GB/T 18654.13-2008. The document is under the responsibility of the Ministry of Agriculture and Rural Affairs. This page is published from the official record of the 2026 edition; the clause text of a standard this recent is not yet in circulation, and the figures, limits and tables it contains are those of the document itself, delivered in full with the English translation.

This document describes the principles, reagents and materials, instruments and equipment, samples, and experimental procedures for the analysis of fish isoenzymes using polyacrylamide gel electrophoresis. And data processing. This document applies to isozyme electrophoresis analysis of fish.

4.Principles Isoenzymes are a group of enzymes in fish that catalyze the same reaction but differ in molecular structure and physicochemical properties. They are classified according to their electrical charge. Due to differences in charge, molecular weight, and structure, isoenzyme components exhibit different migration characteristics in polyacrylamide gel media under the influence of an electric field. The enzyme bands, after catalytic reaction and staining, exhibit different numbers of enzyme bands, serving as biochemical genetic characteristics of fish germplasm.

5 Reagents and Materials Warning

---Acrylamide, N,N'-methylenebisacrylamide or methylenebisacrylamide, tetramethylethylenediamine, phenazine methyl ester sulfate, Nitrotetrazole blue chloride, alpha-naphthyl acetate, and beta-naphthyl acetate are toxic chemicals. Respiratory and skin protection measures should be taken when handling them. Wash your hands after

using it. Unless otherwise specified, all reagents were of analytical grade. The water used in the tests was Grade I water conforming to GB/T 6682.

5.1 Reagents

5.1.1 Glacial acetic acid (CH3COOH).

5.1.2 Ethanol (CH3CH2OH).

5.1.3 Glycerol (C3H8O3).

5.1.4 Sodium hydroxide (NaOH).

5.1.5 Nitrotetrazolium chloride (NBT). Store at 4°C.

5.1.6 Tris(hydroxymethyl)aminomethane (Tris).

5.1.7 Citric acid (C6H8O7).

5.1.8 Ethylenediaminetetraacetic acid (EDTA).

5.1.9 Boric acid (H3BO3).

5.1.10 L-histidine (C6H9N3O2).

5.1.11 Acrylamide (Arc).

5.1.12 N,N'-methylenebisacrylamide or methylenebisacrylamide (Bis).

5.1.13 Tetramethylethylenediamine (TEMED).

5.1.14 Ammonium persulfate (AP).

5.2 Solution Preparation 5.2.1 2.5% glacial acetic acid. Measure

2.5 mL of glacial acetic acid (5.1.1), add

97.5 mL of water, and mix well.

5.2.2 Fixative. 3 parts ethanol (5.1.2), 1 part glacial acetic acid (5.1.1), 1 part glycerol (5.1.3), and 5 parts water, mix well. 5.2.3 5% sodium hydroxide solution. Weigh 5g of sodium hydroxide (5.1.4) and dilute to 100mL with water. 5.2.4 1 mg/mL Nitrotetrazole Blue Chloride Solution. Weigh 100 mg of nitrotetrazole blue chloride (5.1.5) and dilute to the final volume with water. 100mL.

5.2.5 TC gel-forming buffer. Weigh 3.0285 g of tris(hydroxymethyl)aminomethane (5.1.6), adjust the pH to

8.0 with citric acid (5.1.7), and add water. Bring the volume to 1L.

5.2.6 EBT gel preparation buffer. Weigh 5.4513 g of tris(hydroxymethyl)aminomethane (5.1.6) and 0.2922 g of ethylenediaminetetraacetic acid (5.1.8), and use... Adjust the pH to

8.6 with boric acid (5.1.9) and bring the volume to 1L with water.

5.2.7 HC gelation buffer. Weigh 1.9395g histidine (5.1.10) and 21.014g citric acid (5.1.7), and use 5% sodium hydroxide solution. (5.2.3) Adjust the pH to

8.2 and bring the volume to 1L with water.

5.2.8 TC Electrode Buffer. Weigh 4.865g of tris(hydroxymethyl)aminomethane (5.1.6), adjust the pH to

8.0 with citric acid (5.1.7), and dilute with water. Fill to 1L.

5.2.9 EBT Electrode Buffer. Weigh 65.4g of tris(hydroxymethyl)aminomethane (5.1.6) and 3.51g of ethylenediaminetetraacetic acid (5.1.8), and use boric acid... (5.1.9) Adjust the pH to

8.6 and bring the volume to 15L with water.

5.2.10 HC Electrode Buffer. Weigh 1.9395g L-histidine (5.1.10) and 21.014g citric acid (5.1.7), and dissolve in 5% sodium hydroxide. Adjust the pH of solution (5.2.2) to 8.5, and then bring the volume to 10L with water.

5.2.19 Electrode buffer stock solution. Weigh

6.00 g of tris(hydroxymethyl)aminomethane (5.1.6) and

28.8 g of glycine (5.1.18), and use hydrochloric acid... (5.1.15) Adjust to pH 8.3, then bring the volume to 1L with water. Adjust as needed.

5.2.20 Gel reservoir (Arc.Bis solution). Weigh 93.75g acrylamide (5.1.11), 2.5g N,N'-methylenebisacrylamide or methyl methacrylate (MCMA). Add bisacrylamide (5.1.12) and dilute to 250 mL with water. 5.2.21 1750 mg/mL ammonium persulfate solution (prepared fresh). Weigh

17.5 g of ammonium persulfate (5.1.14) and dissolve it in 10 mL of water.

5.2.22 Staining solution. Several common isoenzyme staining solutions are prepared according to Appendix A.

5.3 Materials

5.3.1 Syringe. 5mL.

5.3.2 Centrifuge tubes. 1.5mL in size.

5.3.3 Glass homogenizer.

5.3.4 Glue making mold.

5.3.5 Micro-volume syringe. volume range 1μL~20μL, accuracy 0.3%~1.5%.