

Usage procedure for: **Gram Stain Kit, Traditional**

Avantik Kit Item #: RS4558-125 | RS4558-250 | RS4558-500

Solutions Provided in Stain Kit:

Gentian Violet Stain
Iodine Solution, Gram
Gram's Decolorizer Solution
Safranin O Stain
Picric Acid-Acetone Solution

FILMS - Conventional Procedure

1. Flood films with **Genetian Violet Stain** for **1 minute**.
2. Rinse thoroughly in distilled water.
3. Flood films with **Iodine Solution, Gram** for **1 minute**.
4. Rinse thoroughly in distilled water.
5. Flood films with **Gram's Decolorizer Solution** until color stops running. **Up to 10 seconds** should be sufficient.
6. Rinse thoroughly in distilled water.
7. Flood films with **Safranin O Stain** for **1 minute**.
8. Rinse thoroughly in distilled water.
9. Air dry film and examine microscopically.

TISSUES - Conventional Procedure

1. Deparaffinize and hydrate sections through alcohol to distilled water.
2. Place slides on staining rack and cover sections with **Genetian Violet Stain** for **1-2 minutes**.
3. Wash sections well with distilled water until all excess stain is removed.
4. Mordant sections in **Iodine Solution, Gram** for **5 minutes**.
5. Rinse slides well in several changes of distilled water and blot dry.
6. Decolorize briefly in **Gram's Decolorizer Solution**, **1-3 dips** should be sufficient.
7. Rinse sections quickly in distilled water.
8. Cover sections in **Safranin O Stain** for **30-60 seconds**.
9. Rinse slides well in several changes of distilled water and blot dry.
10. (Optional) Dip in Acetone for 1 dip.
11. Cover sections in **Picric Acid-Acetone Solution** for **2-10 seconds**. Sections should have yellowish-pink color.
12. (Optional) If sections have retained an excessive amount of the Picric Acid-Acetone Solution they may be dipped several times in a 1:1 Acetone-Xylene Solution until proper differentiation has been achieved.
13. Dehydrate sections very quickly in 2 changes of 100% Ethanol.
14. Clear in xylene and mount with appropriate mounting medium.



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Results:

Gram-Positive Organisms:	Blue
Gram-Negative Organisms:	Red
Nuclei:	Red
Background:	Yellow

Kit Components and Related Control Slides:

Avantik Item Numbers:

Gentian Violet Stain: RS4408-250 (250 mL) | RS4408-500 (500 mL)

Iodine Solution, Gram: RS4414-250 (250 mL)

Gram's Decolorizer Solution: RS4413-250 (250 mL) | RS4413-500 (500 mL)

Safranin O Stain: RS4444-250 (250 mL) | RS4444-500 (500 mL)

Picric Acid-Acetone Solution: RS4437-250 (250 mL)

Gram Stain Control Slides: SL6211-A (Pack/12) | SL6211-B (Pack/25) | SL6211-D (Pack/100)