WRIGHT STAIN POWDER

Romanowsky-Type Hematological Stain

Catalog No.	Product Code	CAS No.	HS Code
ASD-625	**ASC-1037**	**68988-92-1**	**3204.19.00**

Issue Date	Version	Review Date	Document No.
01 January 2025	**v1.0 — Initial Issue**	**01 January 2026**	**TDS-ASD625-v1.0**

1. PRODUCT IDENTIFICATION

Product Name	Wright Stain (Powder)
Alternate Names	Wright's Stain · Romanowsky Stain (polychrome) · Wright-Giemsa (when combined)
Catalog Number	ASD-625
CAS Number	68988-92-1
HS / AHECC Code	3204.19.00 — Synthetic organic colouring matter (other)
EC / Index No.	Not assigned (mixture)
Physical Form	Fine powder
Appearance	Dark green to black powder; dissolves to blue-purple solution
Grade	Laboratory / Diagnostic Stain — Hematology Grade
Intended Use	Differential staining of peripheral blood smears, bone marrow aspirates, and cytological preparations
Supplier	AuSaMiCs Pty Ltd · 31 Longview Ct, Thomastown VIC 3074, Australia
Contact	+61 412 520 598 · support@ausamics.com · ausamics.com.au

2. PRODUCT OVERVIEW

Background. Wright Stain is a Romanowsky-type polychrome biological stain, developed by James Homer Wright in 1902 as a refinement of Romanowsky's original methylene blue-eosin mixture. It remains one of the most widely used stains in clinical hematology and cytology worldwide.

Chemistry. The stain is a complex mixture of oxidised methylene blue derivatives — including azure A, azure B, and azure C — combined with eosin Y (an acidic xanthene dye). This polychrome mixture is prepared by precipitation, then dissolved in anhydrous methanol (as the solvent and fixative in working solution preparation).

Staining Mechanism. Differential staining is achieved through electrostatic interactions between the dye components and cellular structures. Basic dyes (azure derivatives) bind to acidic components (nucleic acids, nucleoproteins), while the

acidic dye (eosin) binds to basic components (haemoglobin, eosinophil granule proteins). The pH of the buffer used during staining is critical — optimal results require pH 6.4–6.8.

Clinical Significance. Wright Stain enables clear differentiation of all major blood cell lineages and is used in the diagnosis of haematological disorders, infections, leukaemia, malaria, and other blood-borne parasitic diseases. It is the standard stain in haematology laboratories across Australia, the UK, and the United States.

KEY ADVANTAGE AuSaMiCs Wright Stain is manufactured to hematology grade with consistent dye content ratio, ensuring reproducible polychrome staining results and reliable differential cell counts across all batches.

3. APPLICATIONS

HEMATOLOGY

- Peripheral blood smear staining
- Differential leukocyte counting (5-part diff.)
- Erythrocyte morphology assessment
- Platelet morphology evaluation
- Reticulocyte examination
- Bone marrow trephine and aspirate staining
- Leukaemia / lymphoma morphology

PARASITOLOGY & MICROBIOLOGY

- Detection of Plasmodium spp. (malaria)
- Identification of Trypanosoma species
- Babesia and Theileria identification
- Microfilaria detection
- Intracellular organism identification
- Leishmania amastigote detection
- Blood-borne protozoa screening

CYTOLOGY & RESEARCH

- Fine needle aspiration (FNA) cytology
- Body fluid cell differentials (CSF, pleural)
- Synovial fluid analysis
- Veterinary hematology
- Cell culture morphology assessment
- Chromosome spread staining
- Research and teaching preparations

4. STAINING PRINCIPLE & CHEMISTRY

Stain Classification	Romanowsky-type · Polychrome hematological stain
Dye Components	Methylene blue oxidation products: azure A, azure B, azure C, thionin (basic dyes); Eosin Y (acidic dye)
Solvent / Fixative	Anhydrous methanol — acts simultaneously as solvent and cell fixative during staining
Buffer System	Phosphate or Sörensen's buffer, pH 6.4–6.8 for washing / dilution step
Optimal pH	6.4–6.8 (buffer wash). pH significantly affects staining quality — too acidic = more red; too alkaline = more blue
Fixation Mechanism	Methanol denatures proteins and lipids, fixing cellular morphology without further fixation step required
Staining Principle	Electrostatic (ionic) interaction: basic dyes bind acidic cellular components (nucleic acids); eosin binds basic components (Hb, granule proteins)
Temperature Sensitivity	Staining is more intense at room temperature (20–25°C). Avoid staining in cold rooms.

5. EXPECTED STAINING RESULTS

IMPORTANT Exact colour intensities depend on buffer pH, staining time, dye concentration, and smear thickness. The colours below represent expected results under optimal conditions (pH 6.4–6.8, 20–25°C).

Swatch	Cellular Component	Staining Colour	Dye Interaction
	Nuclei (WBC / granulocytes)	Purple / dark violet	Basic dye (methylene blue) → acidic chromatin
	Nuclear chromatin	Light to mid purple	Polychrome methylene blue derivatives
	Lymphocyte cytoplasm	Pale sky blue	Basic dye → weakly acidic cytoplasm
	Erythrocytes (RBCs)	Pink to salmon-orange	Acidic dye (eosin) → basic haemoglobin
	Eosinophil granules	Bright red / orange-red	Eosin → highly basic granule proteins (MBP)
	Basophil granules	Deep purple / violet	Methylene blue → sulphated proteoglycans
	Platelets	Pale violet / lilac	Light polychrome staining
	Monocyte cytoplasm	Grey-blue / slate	Mixed basic dye interaction
	Toxic / reactive granules	Orange-brown	Altered dye affinity in activated neutrophils
	Plasma cells	Intensely blue cytoplasm	High RNA content — strong basic dye affinity

** MBP = Major Basic Protein (eosinophil granule constituent). Colour swatches are indicative — calibrate against reference slides for critical diagnostic work.*

6. PREPARATION OF WORKING SOLUTION

NOTE Wright Stain powder must be dissolved in anhydrous (absolute) methanol — water must be completely excluded at this stage. Any moisture will cause precipitation and degraded staining quality.

Method A — Standard Stock Solution (Recommended)

Step	Action	Parameters / Notes
1	Weigh 0.3 g of Wright Stain powder accurately on an analytical balance	Use glass or metal weighing vessels — avoid plastic contact
2	Transfer to a clean, dry 100 mL amber glass volumetric flask	Glass only — methanol dissolves many plastics
3	Add 100 mL of anhydrous methanol (≥99.8% purity, water content <0.05%)	Absolute methanol — not denatured or industrial methylated spirits
4	Seal and shake vigorously for 2–3 minutes to begin dissolving	Initial dissolution is slow — do not heat
5	Place on a laboratory roller or rocker shaker at room temperature for 24–48 hours	Complete dissolution required — no undissolved particles
6	Filter through Whatman No. 1 filter paper or 0.45 µm membrane filter	Remove undissolved particles — critical for clean background
7	Transfer to amber glass bottle. Label with: name, concentration, date, initials	Stable at 15–25°C for 12 months. Store tightly sealed away from light.

Method B — Rapid Dissolution (For urgent use)

Step	Action	Parameters / Notes
1	Dissolve 0.3 g Wright Stain in 100 mL anhydrous methanol with gentle stirring on a magnetic stirrer	Do NOT heat above 35°C
2	Stir continuously for 4–6 hours at room temperature until fully dissolved	Monitor for complete dissolution
3	Filter and store as per Method A steps 6–7	Allow to equilibrate 24 h before use for best results

Stock Concentration	3 g/L (0.3%) in anhydrous methanol — standard working concentration
Buffer for Washing	Phosphate buffer pH 6.4–6.8 (Sörensen's buffer or prepared buffer tablets available separately)
Working Dilution	Use undiluted stock for direct staining; dilute 1:1 to 1:3 with buffer for rapid/automated methods
Shelf Life (stock)	12 months at 15–25°C in sealed amber glass bottle, protected from light and moisture
Quality Check	Stock solution should be deep blue-purple. Yellow-green tinge indicates degradation — discard and prepare fresh.

7. STAINING PROTOCOLS

Protocol A — Manual Blood Smear Staining (One-Step)

Step	Action	Parameters / Notes
1	Prepare air-dried peripheral blood smear on clean glass slide. Do not fix separately — methanol in stain acts as fixative.	Smear should be completely dry (minimum 30 min)
2	Place slide horizontally on staining rack	Ensure slide is level to prevent run-off
3	Flood slide with undiluted Wright Stain stock solution (approx. 1–2 mL)	Cover entire smear surface
4	Allow to stand for 1–3 minutes (fixation step)	Time depends on smear thickness — thicker smears need longer
5	Without draining stain, add equal volume of phosphate buffer pH 6.4–6.8	Mix gently by blowing or rocking — a metallic sheen (scum) should appear on surface
6	Allow buffer-stain mixture to act for 5–10 minutes	Optimal time varies by batch — validate with each new lot
7	Rinse slide with buffered water or distilled water (pH 6.4–6.8)	Do NOT use tap water — variable pH causes inconsistent results
8	Drain and allow to air-dry completely in vertical position	Do NOT blot — allow to air-dry
9	Examine under oil immersion (100× objective)	Coverslip with DPX or equivalent mounting medium for permanent preparation

Protocol B — Rapid Staining (Dip Method)

Step	Action	Parameters / Notes
1	Air-dry blood smear completely	Minimum 30 minutes, or 5 min in 37°C incubator
2	Dip slide into neat Wright Stain for 1 minute (10 dips × 3 seconds each)	Consistent dipping motion — keep tempo even
3	Transfer to buffer (pH 6.4–6.8) for 30 seconds (5 dips × 6 seconds each)	Use freshly prepared or verified buffer
4	Rinse gently in buffered distilled water	2–3 second rinse only
5	Drain and air-dry upright	Ready to examine in 5–10 minutes

Protocol C — Wright-Giemsa Combined Stain (Enhanced Morphology)

Step	Action	Parameters / Notes
1	Fix air-dried smear in neat methanol for 2 minutes, then air-dry	Separate fixation step improves nuclear detail
2	Stain in Wright Stain for 2 minutes	Standard stock 3 g/L
3	Without rinsing, flood slide with Giemsa stain diluted 1:20 in pH 6.8 buffer for 15 minutes	Giemsa (AuSaMiCs Cat. No. available separately) enhances nuclear and parasite detail
4	Rinse with buffer pH 6.8, air-dry, examine under oil	Excellent for parasite detection and leukaemia morphology

TROUBLESHOOTING TIP If smears appear too blue: increase wash time or raise buffer pH slightly. If too pink/red: reduce wash time or lower buffer pH. If background is granular/dirty: filter stock solution and check methanol quality.

8. TROUBLESHOOTING GUIDE

Problem	Likely Cause(s)	Corrective Action
Overly blue staining (RBCs blue/purple)	Buffer pH too high; insufficient wash; excess stain time	Lower buffer pH to 6.4; extend wash step; reduce stain time
Overly pink / red staining (pale nuclei)	Buffer pH too low; over-washing; old or degraded stain	Raise buffer pH to 6.8; reduce wash time; prepare fresh stock
Precipitate / granular background	Wet slides; water in methanol; unfiltered stock solution	Ensure dry smear; use absolute methanol; filter stock before use
Pale, washed-out staining	Staining time too short; dilute stock; over-washing	Increase stain time; check stock concentration; reduce wash
Uneven staining across slide	Slide not horizontal; inconsistent flooding; smear too thick	Ensure level rack; flood entire smear; make thinner smear
Nuclear staining absent or faint	pH too acidic; methanol contaminated with water	Re-check buffer pH; replace methanol; fresh stock solution
Eosinophil granules not red	pH imbalance; degraded eosin component	Verify buffer pH 6.4–6.8; prepare fresh working solution
Metallic scum does not form on buffer addition	Insufficient dye concentration; incorrect ratio of buffer to stain	Ensure 1:1 buffer:stain ratio; verify stock concentration
Short shelf life of prepared smears	Inadequate fixation; humid storage conditions	Fix in methanol 2 min; store coverslipped in dry conditions

9. PHYSICAL & CHEMICAL PROPERTIES

Physical State	Solid — fine powder
Colour (dry powder)	Dark green to black
Colour (dissolved)	Deep blue-purple in anhydrous methanol; blue in aqueous buffer
Odour	Slight characteristic odour (methanol when dissolved)
Solubility	Freely soluble in anhydrous methanol; slightly soluble in water; insoluble in non-polar solvents
Absorption Maximum	~ 650–670 nm (azure B component); ~ 515–520 nm (eosin Y component)
Molecular Weight	Not applicable (polychrome mixture; complex dye system)
CAS Number	68988-92-1 (mixture)
Purity	Dye content ≥ 85% (polychrome-validated; azure/eosin ratio controlled)
Loss on Drying	≤ 5% w/w at 105°C
Ash Content	≤ 1.0% w/w
Water Content (KF)	≤ 2.0% w/w (anhydrous methanol dissolution requires low water content)
Stability (powder)	Stable for 3 years if stored sealed, 15–25°C, away from light and moisture
pH of working solution	6.4–6.8 (phosphate buffer-diluted staining solution)

10. QUALITY CONTROL & RELEASE TESTING

BATCH RELEASE Every batch of AuSaMiCs Wright Stain is tested against the following specifications before release. A Certificate of Analysis (COA) is available for each lot number on request.

Test Parameter	Method	Specification	Result
Appearance	Visual inspection	Dark green-black powder	✓ Complies
Dye content	Photometric assay	≥ 85%	✓ Complies
Polychrome ratio	Spectrophotometric	Azure:Eosin within validated range	✓ Complies
Loss on drying	Gravimetric, 105°C/2h	≤ 5.0% w/w	✓ Complies
Water content	Karl Fischer titration	≤ 2.0% w/w	✓ Complies
Solubility in MeOH	Dissolution test	Complete at 3 g/L	✓ Complies
Performance (blood smear)	Reference blood smear staining	All cell types correctly differentiated per colour chart	✓ Complies

Test Parameter	Method	Specification	Result
pH of working sol.	pH meter, calibrated electrode	6.4–6.8	✓ Complies
Ash content	Gravimetric, 550°C/1h	≤ 1.0% w/w	✓ Complies

11. STORAGE & STABILITY

Powder — Temperature	+15°C to +25°C (controlled room temperature)
Powder — Humidity	≤ 60% RH · Keep sealed · Protect from moisture (hygroscopic)
Powder — Light	Store in original amber/dark container · Avoid direct sunlight and UV exposure
Powder — Container	Keep in original tightly sealed container · Re-seal immediately after use
Powder — Shelf Life	36 months from date of manufacture when stored correctly. Refer to label for expiry date.
Stock Solution (MeOH)	12 months in amber glass, sealed, at 15–25°C away from light. Label with date prepared.
Working Solution	Prepare fresh or use within 1 week. Discard if colour changes or precipitate appears.
Prepared Slides	Coverslipped permanent slides stable indefinitely if stored in dry, dark conditions.
Stability Indicator	Fresh stock: deep blue-purple. Degraded stock: yellow-green or pale. Discard degraded solutions.

12. PACKAGING & ORDERING INFORMATION

Pack Size	Catalog No.	Approx. Slides (3 g/L stock)	Suitable For
10 g	ASD-625-10	~330 slides	Small labs, evaluation
25 g	ASD-625-25	~830 slides	Routine laboratory
100 g	ASD-625-100	~3,300 slides	High-volume laboratory
500 g	ASD-625	~16,500 slides	Hospital / reference lab
Bulk	ASD-625-BLK	Custom	Commercial / OEM use

** Slide yield based on 1 mL stock per slide. Actual yield depends on application method and operator technique. Contact AuSaMiCs for bulk pricing and custom quantities.*

13. RELATED PRODUCTS & ACCESSORIES

Giemsa Stain (Powder)	AuSaMiCs Cat. No. ASD-610 · Used with Wright for Wright-Giemsa combined protocol

Giemsa Stain (Solution)	AuSaMiCs Cat. No. ASD-611 · Ready-to-dilute working solution
Leishman Stain	AuSaMiCs Cat. No. ASD-630 · Alternative Romanowsky stain for blood films
May-Grünwald Stain	AuSaMiCs Cat. No. ASD-640 · Complement to Wright for bone marrow and differential
Phosphate Buffer Tablets pH 6.8	AuSaMiCs Cat. No. ABF-100 · Convenient buffer preparation for staining wash step
Anhydrous Methanol	Available from AuSaMiCs or approved solvent supplier · ≥99.8% purity required
DPX Mountant	AuSaMiCs Cat. No. AMT-200 · For permanent coverslipping of stained smears
Certificate of Analysis	Available for each lot on request · Email: support@ausamics.com
Safety Data Sheet (SDS)	SDS-ASD625-v1.0 · Available at ausamics.com.au or on request

14. REGULATORY & COMPLIANCE INFORMATION

Intended Use	For laboratory and research use only. Not for in vivo diagnostic, therapeutic, human, veterinary, food, or pharmaceutical use.
AICIS Status	Assessed as exempt from industrial chemical registration for laboratory use quantities (Industrial Chemicals Act 2019, Aust.)
GHS / SDS	Safety Data Sheet available: SDS-ASD625-v1.0. Not classified as hazardous under GHS Rev. 9.
HS / AHECC Code	3204.19.00 — Synthetic organic colouring matter, other
REACH (EU reference)	CAS 68988-92-1 not currently listed as SVHC. Not subject to REACH (Australian product).
Quality Standard	Manufactured under controlled conditions. Batch-tested against validated release specifications.
Document Control	TDS-ASD625-v1.0 · Issue Date: 01 January 2025 · Review: 01 January 2026

AuSaMiCs Pty Ltd · Excellence in Life Science Reagents & Culture Media
31 Longview Ct, Thomastown VIC 3074 · ABN 56 676 640 467 · ausamics.com.au · support@ausamics.com