KAISER PERMANENTE®
COLORADO LABORATORY

# DIFF-QUICK STAIN SET

**PRINCIPLE:**
Diff-Quick Stain Set is a modification of the Wright Stain technique which essentially converts the classical 4 minute, 3 step process into a 15 second staining operation. Blood smears are fixed using the methanolic fixative solution to stabilize cellular components. Solutions I and II are then applied individually to the fixed smear to differentially stain specific cellular components. Solution I is a buffered solution of Eosin Y (an anionic dye). Solution II is a buffered solution of thiazine dyes (cationic dyes) consisting of methylene blue and Azure A. The resultant basophilic staining of nucleoli and cytoplasm is due to the methylene blue component of the mixture. The anionic component of the nucleoli and cytoplasm, which is stained with the cationic methylene blue, is presumed to be RNA. The nuclei are stained red to purple (metachromatically) by the azure component of the dye mixture. It is believed that DNA is capable of functioning as a chromotrope for Azure A, B, and methylene violet. The xanthene solution (I) stains cytoplasm pink to yellowish-red

**SCOPE:**
Medical Technologists and Medical Laboratory Technicians

**SPECIMEN REQUIREMENTS AND HANDLING:**
Fresh venous blood collected in EDTA is the specimen of choice. If the smear is not made immediately, mix the blood by gentle inversion 10-15 times. Blood can be stored at 2-8° C until used but ensure that blood is at room temperature and mixed well prior to making the peripheral blood smear.

**EQUIPMENT AND MATERIALS:**
1. Slides
2. Diff Safe device
3. Coplin Jars with lids
4. Diff Quick Solution I – stored at room temperature (15-30° C). Do not use past the expiration date.
5. Diff Quick Solution II - stored at room temperature (15-30° C). Do not use past the expiration date.
6. Water – Tap or DI (NERL) water is acceptable

**QUALITY CONTROL:**
1. The stain quality is checked once per day when patient manual differential is performed. Make two peripheral blood smears on the first manual differential of the day and label one slide as "QC" with the date and the other slide with the patient information according to the procedure. The following items are verified on the QC slide:
    a. WBC stained correctly
    b. RBCs stained correctly
    c. Platelets stained correctly
    d. Stain quality is appropriate for performing manual differential – not too light or dark.

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**NOTE**: If stain quality is not acceptable, discard solutions I and II and pour new reagents into the coplin jars.

2. Record stain quality on the daily Diff Quick maintenance log and mark with an "X" when there is no manual differential performed that day.

**PROCEDURE:**

1. Prepare blood smear and allow to dry.
2. Dip slide in Fixative Solution Five times, one second each time. Allow excess to drain.
3. Dip slide in Solution I Five times, one second each time. Allow excess to drain.
4. Dip slide in Solution II Five times, one second each time. Allow excess to drain.
5. Rinse slide with distilled or deionized water.
6. Allow slide to dry. Examine under dry low power and oil immersion objectives.

**NOTE:**

- If a more intense overall stain is desired, increase the number of dips in Solutions I and II. If a paler stain is desired, decrease dips in Solutions I and II, but never go below three dips of one full second each.
- To increase eosinophilic staining, increase the number of dips in Solution I. To increase basophilic staining, increase the number of dips in Solution II.
- Coplin jars should be kept tightly covered when not in use.

**MAINTENANCE:**

1. Solutions I and II are changed once per week. It may be necessary to change one or both solutions more frequently if stain quality is not acceptable. Document this on the maintenance log.
2. Water is changed daily and more frequently if stain quality is not acceptable.

**REPORTING RESULTS:**

Refer to the Manual Differential procedure

**LIMITATIONS OF THE PROCEDURE:**

Good technique in preparing the blood smears should be used at all times. The unique division of stains in the Diff-Quick Set gives the user the advantage of varying dips in Solutions I and II to produce different degrees of shading and intensity. However, one must never use fewer than three dips of one full second each. Diff-Quick is an aqueous stain, and the water soluble portions of cell material may take the stain differently than the classical alcohol-based Wright Stain.

**DISTRIBUTION:**

Medical Office Laboratories that are not using the Hematek Automated Slide Stainer

**REFERENCES:**

Dade Behring Diff Quick Stain Set Package Insert (Revision Date 03/02)

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