

Protocol***Histochemical staining of granulocytes (neutrophils and macrophages)*****Equipment to Be Supplied by User**

- Makler counting lam
- Pipettor
- Dark microcentrifuge tube

Some tips to know

- SSB is yellow, in case of any color change; please contact technical support team.
- It is better to prepare working solution freshly. The shelf life of this solution is one week in dark environment at room temperature (15-25°C).

Preparing Working Solution for 5 Reactions

- Blend 200 µl SSB with 2.5 µl HRB in clean dark tube or use transparent tube and cover it with foil. Then, pulse vortex for 5s to homogenize the solution. Shelf life of this solution is one week in dark environment at room temperature.

Process

1. Transfer 20 µl liquefied semen specimen in dark colored microcentrifuge tube.
2. Add 20 µl PBS, 1X to previous step; then, add 40 µl working solution.
3. Vortex and incubate at room temperature for 5 min.
4. Load a Makler counting chamber with 5 µl of the above solution and observe under a 10× bright-field objective lens.
5. Identify the granulocyte with dark brown color and sphere shape. Count the cells in all 100 squares of the Makler grid.

Calculation

Calculate the percentage of leukocyte in semen specimen by this formula:

$$\text{Millions of leukocyte /mL} = [\text{Counted WBC} \times 4 \text{ (dilution factor)}] / 10$$

Reference range

$<0.20 \times 10^6/\text{mL}$ (normal): Routine semen analysis and semen profile

$<1.0 \times 10^6/\text{mL}$ (normal): IUI samples and basic semen analysis

Example

- 5 WBCs are counted in 100 squares on the Makler grid.
- positive cells = $\text{WBC} \times 4/10 = 5 \times 0.40 = 2.0 \text{ M/ml}$.
- Report results as million/ml.
- A normal concentration of white blood cells in semen is $< 0.20 \times 10^6/\text{ml}$, and therefore anything greater or equal to $0.20 \times 10^6/\text{mL}$ will be considered as significant.