

Oil Red O solution

Cat #: orb1566682 (manual)

Size: 20 mL, 100 mL, 500 mL

Product Composition

Reagent name	orb1566682-1	orb1566682-2	orb1566682-3
Oil Red O solution	20 mL	100 mL	500 mL
Manual	1 copy	1 copy	1 copy

Product Introduction

Lipid is a general term for neutral fats, lipids and their derivatives, which are insoluble in water and soluble in organic solvents. There are two main types of lipids in the human body: one is the storage of lipids, such as neutral fats (i.e., triglycerides), which are mainly distributed in the subcutaneous, kidney, pancreas and other parts; The second is structural lipids, such as lipids (phospholipids, glycolipids, cholesterol, etc.), which are mainly distributed in cells. Lipid droplets are the main storage site of neutral fat in cells, under normal circumstances, except for fat cells, other cells are almost invisible under the light microscope, if a large number of lipid droplets appear in the cytoplasm, it indicates steatosis, commonly found in hepatocytes, cardiomyocytes, renal convoluted epithelial cells, etc.

Oil Red O is a fat-soluble azo dye, which is a strong lipid solvent and staining agent, which can specifically stain tissues and cells with neutral triglycerides, lipids, lipoproteins, etc., and the effect is better than the traditional Sudan II., Sudan III., Sudan IV., Sudan Black B, etc. When stained with Oil Red O, the solubility of Oil Red O in lipids in tissue cells is greater than in the original solvent, so Oil Red O is transferred from the organic solvent to the lipid to stain it.

Oil Red O Solution can reflect the steatosis and abnormal lipid deposition of tissues and organs by showing lipid droplets in cells. It is also possible to identify tumors that occur in adipose tissue and their nature. This product can be used both for tissue staining and for the analysis of lipid profiles in cell samples. Depending on the concentration of lipids, positive lipid staining results can be orange to red. This product is made of high-quality imported raw materials, with clear and stable dyeing and convenient operation. 100ml of Saturated Oil Red O staining solution can be prepared into approximately 166ml of working solution, and 500ml of Saturated Oil Red O staining solution can be prepared into approximately 833ml of working solution.

Storage conditions

Store at 2-8°C in the dark, valid for one year.

Operation Procedure

Prepare the working solution: According to the ratio of saturated oil red O staining solution: distilled water = 3:2, prepare oil red O working solution, mix well, place at room temperature for 5-10min, and use after filtration. **【Oil Red O dyeing solution is unstable and should not be prepared in advance】** .

Dyeing procedures

1. Frozen slices:

(1) The prepared frozen sections are not fixed or fixed with 10% neutral formalin for 10min, and washed fully with distilled water.

(2) 60% isopropanol immersion for 20-30s.

(3) Oil red O dyeing working solution dyeing for 10-15min. [The time can be adjusted according to the dyeing results and requirements]

(4) 60% isopropanol differentiates to clear interstitium and is slightly cleaned in distilled water.

(5) Mayer hematoxylin staining solution counterstained nuclei for 1-5min. [The time can be adjusted according to the dyeing results and requirements]

(6) (Optional) 1% hydrochloric acid solution to differentiate slightly.

(7) (Optional) Rinse with tap water for 10min or promote blue in dilute lithium carbonate solution.

(8) Wash slightly in distilled water.

(9) Blot the surrounding water with filter paper, cover the sheet with glycerin gelatin and observe under a microscope.

2. Culturing Cells:

(1) Remove the cell culture medium, wash twice in PBS, and fix with 10% neutral formalin fixative solution for 20-30min.

(2) Discard the fixative solution and wash 2 times with distilled water.

(3) Immersion in 60% isopropanol for 5min.

(4) After discarding 60% isopropanol, add oil red O staining solution, cover the bottom plate, and dip for 10-20min. [The time can be adjusted according to the dyeing results and requirements]

(5) Discard the staining solution and wash 2-5 times with distilled water until there is no excess staining solution.

(6) Mayer hematoxylin staining solution counterstained nuclei for 1-2min. **【The time can be adjusted according to the staining results and requirements】** After discarding the dyeing solution, wash it with water 2-5 times.

(7) Differentiate with 1% hydrochloric acid solution for 1min and discard.

(8) Add distilled water to cover the cells and observe under the microscope.

3. Cell smear

- (1) Prepare fresh bone marrow and blood smears, and fix them in 10% neutral formalin for 10-15min.
- (2) Take out the smear and put it in the circulating air for 10-15min.
- (3) Put in the oil red O dyeing solution for 15min. [The time can be adjusted according to the dyeing results and requirements]
- (4) 60% isopropanol differentiation for 20-30s, rinse with running water, and wash slightly with distilled water.
- (5) Mayer hematoxylin staining solution counterstained nuclei for 1-2min. [The time can be adjusted according to the dyeing results and requirements]
- (6) Differentiate with 1% hydrochloric acid solution for 1min, and wash slightly with distilled water.
- (7) Blot the surrounding water with filter paper, mount the sheet with glycerin gelatin and observe under the microscope.

Staining results

Neutral fats	Orange to red
Phospholipids	Pink
Nucleus	Blue

Precautions

1. Oil Red O dyeing solution is not stable enough and easy to precipitate, so it is not suitable for preparation in advance. The working fluid can be reused, but if it is not used up on the same day, it needs to be discarded.
2. Organic solvents dissolve lipids in the sample, so fixation of the sample is not done with a fixative solution containing ethanol (10% neutral formalin or 10% formalin if fixation is required), nor is paraffin sectioning or carbonwax sectioning required.
3. If you do adipose tissue frozen sections, the freezing temperature is lower than normal, and the thickness of the sections should not be too thin, because too thin sections often cause lipid loss, and the general thickness is 6-10µm.
4. Fully mature and plump fat cells are easy to break down and lipid droplets leak, so attention should be paid when pressing and slicing.
5. Samples of glycerin gelatin mounts are not stored for a long time. For long-term storage, the edge of the coverslip-slide junction can be closed with neutral gum.

6. Excessive volatilization of the reagent should be avoided when staining with oil red O, otherwise it is easy to form background precipitation. When staining, the staining solution should adequately cover the tissue cells.
7. 60% isopropanol differentiation can be controlled under the microscope until the adipose tissue is bright red and the interstitium is colorless. Hematoxylin counterstaining is recommended to increase the contrast of staining.
8. When using this reagent for the first time, it is recommended to take 1-2 samples for pre-experiment.
9. This reagent is a saturated solution of oil red O, so a small amount of precipitation is normal. When using, it should be filtered by itself after being prepared into a working solution.
10. For your safety and health, please wear a lab coat and disposable gloves.