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# ab228563 7-AAD Staining Solution

For the exclusion of non-viable cells in flow cytometry

This product is for research use only and is not intended for diagnostic use.

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# 1. Overview

7-Amino-Actinomycin D (7-AAD) is ready-to-use nucleic acid dye solution (2 ml). 7-AAD can be used in place of propidium iodide (PI) for the exclusion of non-viable cells in flow cytometry analysis. This product can be used in combination with PE (phycoerythrin), and FITC (Fluorescein isothiocyanate) conjugated antibodies in 2-color analysis. The advantage of 7-AAD over PI is the minimal spectral overlap between these emissions. Fluorescence is detected in the far-red range of the spectrum (650 nm long-pass filter).

Harvest cells by centrifugation at 300 x g for 5 minutes.



Remove supernatant



Wash cells in 2 ml PBS/BSA and centrifuge at 300 x g for 5 minutes



Resuspend in 100  $\mu$ L PBS



Add 5  $\mu$ L 7-AAD to cells. Mix well and incubate 5 minutes at room temperature



Add 300  $\mu$ L PBS



Analyze by flow cytometry

## 2. Materials Supplied and Storage

Store 7-AAD at 4°C in the dark immediately on receipt.

### 3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Centrifuge for harvesting/washing cells.
- Flow cytometer capable of measuring fluorescence at Ex/Em = 540/645 nm.

## 4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:

[www.abcam.com/assaykitguidelines](http://www.abcam.com/assaykitguidelines)

**ΔNote:** This product contains sodium azide. In acid conditions, it is transformed into hydrazoic acid, a highly toxic compound. Azide compounds must be diluted in running water before being discarded. These conditions are recommended so as to avoid deposits in plumbing, where explosive conditions could develop.

## 5. Reagent Preparation

### 5.1 7-AAD

Ready to use as supplied.

Sufficient reagent is provided for 400 tests (at 5  $\mu\text{L}$ /test).

## 6. Assay Procedure

All cell staining procedures should be performed under sterile conditions, such as in a laminar flow hood.

- 6.1 Harvest cells (corresponding to  $2 \times 10^5$  to  $1 \times 10^6$  cells). Centrifuge the cells at  $300 \times g$  for 5 minutes and remove the supernatant. Resuspend the pellet in the residual liquid.
- 6.2 Wash cells once in 2 mL PBS containing 2% BSA. Centrifuge the cells at  $300 \times g$  for 5 minutes and remove the supernatant. Resuspend the pellet in 100  $\mu$ L PBS.
- 6.3 Add 5  $\mu$ L of 7-AAD to the resuspended cell pellet and mix well. Incubate at room temperature for 5 minutes before analysis.
- 6.4 After the 5-minute incubation period, add 300  $\mu$ L PBS. Analyze by flow cytometry. Excitation = 540 nm / emission = 645 nm.

**ΔNote:** Before acquiring samples, adjust the discriminator (threshold) to minimize debris.

Fluorescence is detected in the far red range of the spectrum (650 nm long-pass filter).



## 7. Notes





## Technical Support

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### **Austria**

wissenschaftlicherdienst@abcam.com | 019-288-259

### **France**

supportscientifique@abcam.com | 01.46.94.62.96

### **Germany**

wissenschaftlicherdienst@abcam.com | 030-896-779-154

### **Spain**

soportecientifico@abcam.com | 91-114-65-60

### **Switzerland**

technical@abcam.com

Deutsch: 043-501-64-24 | Français: 061-500-05-30

### **UK, EU and ROW**

technical@abcam.com | +44(0)1223-696000

### **Canada**

ca.technical@abcam.com | 877-749-8807

### **US and Latin America**

us.technical@abcam.com | 888-772-2226

### **Asia Pacific**

hk.technical@abcam.com | (852) 2603-6823

### **China**

cn.technical@abcam.com | 400 921 0189 / +86 21 2070 0500

### **Japan**

technical@abcam.co.jp | +81-(0)3-6231-0940

### **Singapore**

sg.technical@abcam.com | 800 188-5244

### **Australia**

au.technical@abcam.com | +61-(0)3-8652-1450

### **New Zealand**

nz.technical@abc.com | +64-(0)9-909-7829