NEUROMICS

JAM-A Data Sheet

Catalog Number:	GT15249	**Host:**	Goat
Product Type:	Affinity purified	**Species Reactivity:**	Human
Immunogen Sequence:	Mouse myeloma cell line NS0-derived recombinant human JAMA Ser28Ala242 Accession # Q9Y624	**Format:**	Liquid 1mg/ml Solution in phosphate-buffered saline (PBS) with 5% Trehlose
Applications:	Western Blot 0.1 µg/mL Immunocytochemistry 5-15 µg/mL Immunohistochemistry 5-15 µg/mL		
	Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Antibody can be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. The antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. *Avoid repeated freeze-thaw cycles.*		

Application Notes

Many cultured cell types do not adhere well to glass coverslips. Adding a thin layer of gelatin to a coverslip enhances the adhesion of cultured cells to glass.

Reagents Required

- Gelatin-coating solution: 0.1% gelatin in deionized H_2O

Materials Required

Coverslips (sterilized)

- Cell culture plate (6- or 24-well)

Procedure

1. Place sterilized coverslips into the wells of a 24-well plate.
2. Add 400 µL of the gelatin-coating solution and incubate the coverslips for 10 minutes at room temperature.
3. Remove the gelatin-coating solution, and air dry the coverslips for 15 minutes.
4. The dried coverslips can now be stored at room temperature until use.

Protocol for the Preparation & Fixation of Cells on Coverslips

Reagents Required

- 1X PBS: 0.145 M NaCl, 0.0027 M KCl, 0.0081 M Na2HPO4, 0.0015 M KH2PO4, pH 7.4
- Formaldehyde Fixative Solution: 2-4% paraformaldehyde in PBS
- Wash buffer: 0.1% BSA in 1X PBS
- Cell culture medium

Materials

- Gelatin-coated coverslips in a 24-well plate

Procedure

1. Culture cells by adding 500 µL of culture media containing approximately 5000 cells to the wells of a cell culture plate containing gelatin-coated coverslips.
2. When cells have reached the desired density/age, remove the culture media from each well and wash twice with PBS.
3. Add 300-400 µL of 2-4% Formaldehyde Fixative Solution to each well, and incubate for 20 minutes at room temperature.

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4. Note: Some cell types can be damaged by the change in surface tension that occurs when the culture medium is entirely removed and replaced with wash buffer. If this is the case, pre-fix the cells by adding 500 µL of 4% Formaldehyde Fixative Solution directly into the culture medium. After 2 minutes, replace the pre-fixation culture medium with 300-400 µL of 2% Formaldehyde Fixative Solution and incubate, for 20 minutes at room temperature.
5. Wash the wells twice with PBS and cover with 400 µL of wash buffer. The coverslips can be stored at 2-8 °C for up to 3 months or they may be stained immediately.
6. Note: Fixation can result in hydrophobic cross-linking of tissue proteins. The time, temperature, pH, and fixative used will determine the degree of cross-linking. Once the fixation protocol has been optimized, the same procedure should be used consistently.

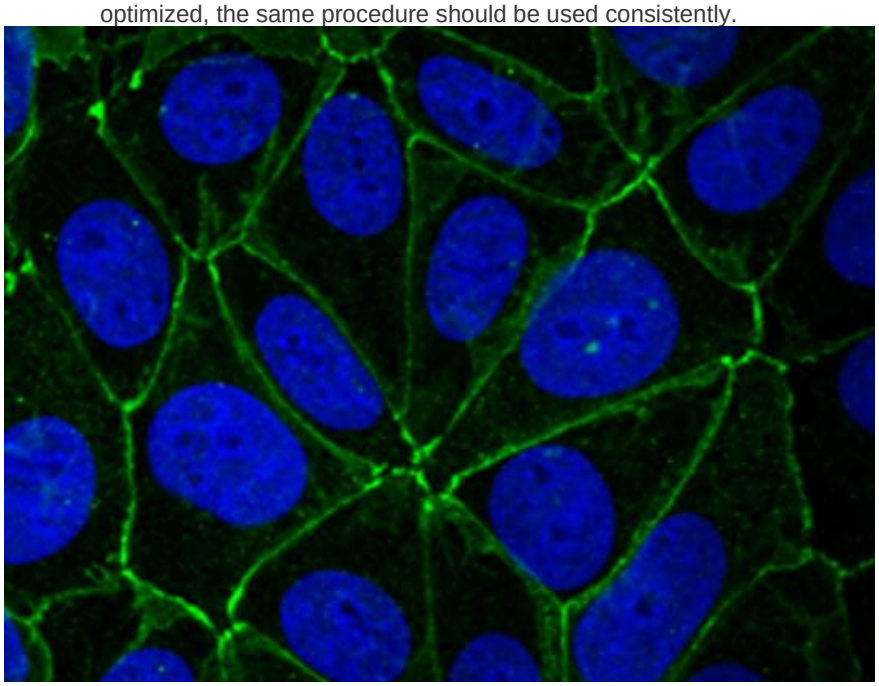

Image: JAM-A was detected in immersion fixed MCF-7 human breast cancer cell line using Goat Anti-Human (green) and DAPI (blue).

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