NEUROMICS

SSEA-3 Data Sheet

Catalog Number:	RA25027	**Host:**	Rat
Product Type:	Rat IgM Monoclonal Antibody	**Species Reactivity:**	Mouse, Human
Immunogen Sequence:	4-8 cell stage mouse embryos.	**Format:**	Liquid. Buffer: PBS 0.02% Sodium Azide as a preservative. Concentration: 1 mg/ml.

Applications: Immunocytochemistry 1:50 - 1:100
Flow Cytometry: 1:50
Immunoprecipitation* Western Blot*
*Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.

Storage: Store at -20°C. Antibody may have become trapped in top of vial during shipping. Centrifugation of vial is recommended before opening. Stable for at least 6 months at -20°C. Repeated freeze/thaw cycles compromise the integrity of the antibody

Application Notes

Specificity: This antibody reacts with the Stage-specific embryonic antigen-3 (SSEA-3) that is expressed upon the surface of human teratocarcinoma stem cells (EC), human embryonic germ cells (EG) and human embryonic stem cells (ES). No immunoreactivity is evident with undifferentiated murine EC, ES and EG cells. Expression of SSEA-3 is down regulated following differentiation of human EC cells. In contrast, the differentiation of murine EC and ES cells may be accompanied by an increase in SSEA-3 expression.

Immunocytochemistry:
Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature. 5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

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