DEVELOPMENTAL STUDIES HYBRIDOMA BANK
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F5D

INVESTIGATOR

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IMMUNOGEN

Substance
- **Name** myogenin
- **Origin** rat
- **Chemical Composition** glutathione-s-transferase fusion protein

Developmental Stage no known isoforms

IMMUNIZATION PROTOCOL

Donor Animal
- **Species** mouse
- **Strain** BALB/c
- **Sex**
- **Organ and tissue** popliteal lymphocytes

Immunization
- **Dates immunized** May 19 & 22 (RIBI), May 26, 30, June 2 & 5 (PBS) 1989
- **Amount of antigen** 50μg Gst-myogenin per site
- **Route of immunization** rear foot pads
- **Adjuvant** RIBI for 1st two injections, then in PBS

FUSION

Date

Myeloma cell line
- **Species** mouse
- **Designation** NS1

MONOCLONAL ANTIBODY

Isotype IgG1, kappa light chain

Specificity
- **Cell binding** no (antigen is internal)
- **Immunohistology** yes
- **Antibody competition** untested

Species Specificity mammalian (tested on rat, mouse, cat, human; quail did not react)

ANTIGEN

Chemical properties rat myogenin

Molecular weight ~ 25 kDa; migrates as ~ 34 kDa on SDS gels; 224 a.a.

Characterization
- **Immunoprecipitation** immunoprecipitates deletion mutants containing a.a. 138-158, a region immediately carboxy-terminal to the bHLH domain.
- **Immunoblotting** positive
- **Purification** cloned
- **Amino acid sequence analysis** deduced from cDNA

Functional effects skeletal muscle-specific bHLH transcription factor probably controlling myoblast to myotube transition.

Immunohistochemistry nuclear

PUBLICATIONS :

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ACKNOWLEDGMENTS STATEMENT

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F5D

Anti-rat Myogenin, monoclonal antibody, lab name originally 1F5D7, renamed F5D for short.
Heavy chain: IgG1 Light chain: K

Epitope: between aa 144-170 (Helix 2 ends at aa 132, the last of the three leucines in the potential leucine zipper is aa 146)

Our growth conditions: RPMI1640 + 10% FCS + 25 μg/ml gentamicin

Our harvest conditions: Seeds cells at 1 million/ml in either HB101 (for serum free) or 2% FCS: Harvest medium q24 hours for 2-3 days until cells have died, counting and reseeding at 1 million/ml each time.

To Freeze: (if cells sent): resuspend cells at one million/ml in 90% serum/10% DMSO. Put inside a cardboard box (like a cell inventory freezer box, approx. 12 cm x 12 cm x 5 cm) and put directly at -80°C overnight or longer, then transfer to -135°C or below.

Known efficacy:

Immunofluorescence: Cells fixed 20 min at room temp in 2% paraformaldehyde in PBS, then permeabilized 10 min at room temp with 0.15% triton X-100 in PBS. We've only done it with neat culture supernatant, but it probably would work if diluted some.

Western Blotting: Good signal with 1:10 diluted culture supernatant.

Gel Shift: Gives good double-shift of myogenin in nuclear extracts using 0.1 μl of a 50-fold concentrated supernatant (i.e., the equivalent of 5 μl of supernatant. Less might still work, but that was the lowest we tried).

Conditions for acceptance of this reagent:
1) Use them for anything you want, but please let me know anything interesting that you find. If you are using them for something other than as a marker etc. that you think is likely to be in direct competition with what I'm doing, it would be nice (but not required) to let me know and we'll discuss whether or not it should be collaborative etc.
2) If you make a derivative reagent (i.e., you purify the antibody and directly label it with anything [fluorescein, rhodamine, HRP, gold, etc]), let me know and agree to provide me with an aliquot if it would be useful to me.
3) You may provide the cells/supernatants to third parties as long as you provide a copy of this letter giving the conditions of use.

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