ANTIBODY TESTING RESULTS

Standard Reporting Template

INSTRUCTIONS: Please complete this form in its entirety. **Providing only a reference publication will not be accepted.**

Date: 25-11-2015

Laboratory Name: Trost

Your Name: Anetta Svitorka Hartlova

Is testing ongoing such that you are waiting for future bleeds?: ☐ Yes ☒ No

Antibody Name: GST-RAB7A

Full Antigen Name: GST-RAB7A (mouse) [DU 46688]

Full Antigen Sequence *(please include full amino acid sequence)*:

GPLGSPGIPGSTRAAAMTSRKKVL LKVIILGDSGVGKTSLMNQYVNKKFSNQYKATIGADFLTKEVMVDDRLVT
MQIWDTAGQERFQSLGVAFYRGADCCVLVFDVTAPNTFKTLDSWRDEFLI
QASPRDPENFPFVVLGNKIDLENRQVATKRAQAWCYSKNNIPYFETSAKE
AINVEQAFQTIARNALKQETEVELYNEFPEPIKLDKNDRAKASAESCSC

Antigen Species *(Please indicate whether the antigen corresponds to the human/mouse/rat or other species)*: Mouse

Bleeds Tested In this Report *(Please check ALL those that apply)*:

☑ 1 ☑ 2 ☑ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

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SUCCESSFUL APPLICATIONS:

Instructions: Please check each box below and indicate clearly all the applications that each bleed was tested in and if it was successful

	Immunoblot		Immunoprecipitation		Immunofluorescence	
	Tested	Successful	Tested	Successful	Tested	Successful
Bleed #1	✓	✓	☐	☐	✓	✓
Bleed #2	✓	✓	☐	☐	✓	✓
Bleed #3	✓	✓	☐	☐	✓	✓
Bleed #4	☐	☐	☐	☐	☐	☐
Bleed #5	☐	☐	☐	☐	☐	☐
Bleed #6	☐	☐	☐	☐	☐	☐
Bleed #7	☐	☐	☐	☐	☐	☐

BEST Working Bleed: All bleeds were fine

PUBLICATIONS: *Please identify all publications to-date that include data supporting the successful use of the antibody*

1. Name, et al, Year, Title, Journal
   - PMID *(mandatory)*
2. Name, et al. (*submitted*)

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SUGGESTED BEST PRACTICES FOR ANTIBODY TESTING

Minimal Dataset

- Overexpressed Protein
  - Recombinant
    - Positive Control – Recombinant protein loaded in a well
    - Negative Control – Mutant recombinant protein
      - Point mutation for phospho-site
      - Truncation mutant that does not contain epitope on antigen used for antibody generation
  - Transfected Cell Lines
    - Positive Control
      - Cell line transfected with construct containing epitope of interest
      - Cell line treated with appropriate compound to illustrate presence of epitope
      - Recombinant protein loaded in a well
    - Negative Control
      - Untransfected cell line (that does not contain protein of interest)
      - Cell line transfected with mutant protein
        - Point mutation for phospho-site
        - Truncation mutant that does not contain epitope on antigen used for antibody generation

Additional Data *(Ideal)*

- Endogenous Protein
  - Cell Lines
    - Positive Control
      - Cell line that endogenously expresses protein
      - Recombinant protein loaded in a well
    - Negative Control
      - Knockout cell line
      - Knockdown of target
        - Genetic
        - Pharmacologic
  - Tissue Homogenate (from relevant source)
    - Positive Control – Tissue source that endogenously expresses protein of interest
    - Negative Control – Same tissue source derived from knockout animal

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IMMUNOBLOT -- DATA

Please include ALL data that illustrates the utility of this antibody:

- All bleeds
- All applications tested:
    - Immunoblot
    - Immunoprecipitation
    - Immunofluorescence

Please ensure that the the following data is included in your figure:

- Positive control
- Negative control

IMMUNOBLOT -- ASSOCIATED FIGURE LEGENDS

Please include ALL text that describes the utility of this antibody for the associated data above:

- All bleeds
- All applications tested:
    - Immunoblot
    - Immunoprecipitation
    - Immunofluorescence

Please ensure that the the following data is included in your description:

- Positive control
- Negative control

IMMUNOBLOT -- EXPERIMENTAL DESIGN

Please include ALL text that describes the utility of this antibody for the associated data above:

- All bleeds
- All applications tested:
    - Immunoblot
    - Immunoprecipitation
    - Immunofluorescence

Please ensure that the the following data is included in your description:

- Positive control
- Negative control

ANTIBODY TESTING RESULTS

Standard Reporting Template

IMMUNOPRECIPITATION -- DATA

Please include ALL data that illustrates the utility of this antibody:

- All bleeds
- All applications tested:
    - Immunoblot
    - Immunoprecipitation
    - Immunofluorescence

Please ensure that the the following data is included in your figure:

- Positive control
- Negative control

IMMUNOPRECIPITATION -- ASSOCIATED FIGURE LEGENDS

Please include ALL text that describes the utility of this antibody for the associated data above:

- All bleeds
- All applications tested:
    - Immunoblot
    - Immunoprecipitation
    - Immunofluorescence

Please ensure that the the following data is included in your description:

- Positive control
- Negative control

IMMUNOPRECIPITATION -- EXPERIMENTAL DESIGN

Please include ALL text that describes the utility of this antibody for the associated data above:

- All bleeds
- All applications tested:
    - Immunoblot
    - Immunoprecipitation
    - Immunofluorescence

Please ensure that the the following data is included in your description:

- Positive control
- Negative control

MRC Protein Phosphorylation and Ubiquitylation Unit

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University of Dundee

IMMUNOFLUORESCENCE -- DATA

Please include ALL data that illustrates the utility of this antibody:

- All bleeds
- All applications tested:
    - Immunoblot
    - Immunoprecipitation
    - Immunofluorescence

Please ensure that the the following data is included in your figure:

- Positive control
- Negative control

IMMUNOFLUORESCENCE -- ASSOCIATED FIGURE LEGENDS

Please include ALL text that describes the utility of this antibody for the associated data above:

- All bleeds
- All applications tested:
    - Immunoblot
    - Immunoprecipitation
    - Immunofluorescence

Please ensure that the the following data is included in your description:

- Positive control
- Negative control

IMMUNOFLUORESCENCE -- EXPERIMENTAL DESIGN

Please include ALL text that describes the utility of this antibody for the associated data above:

- All bleeds
- All applications tested:
    - Immunoblot
    - Immunoprecipitation
    - Immunofluorescence

Please ensure that the the following data is included in your description:

- Positive control
- Negative control

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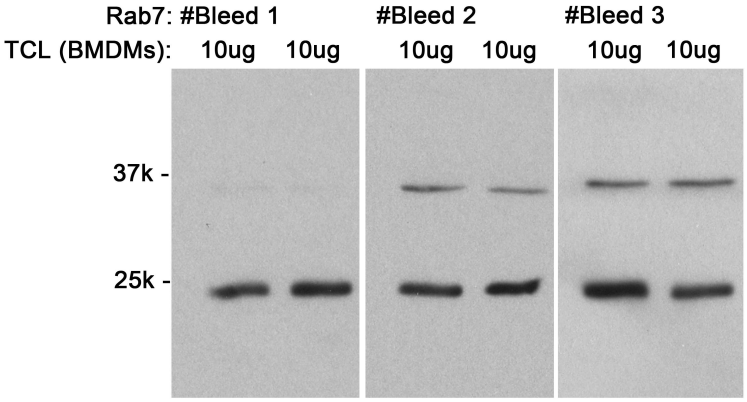
Experiment: #150306

Aim: To test Rab7 Ab (Bleed 1, 2, 3) produced by DSTT

Ab: GST-Rab7A (mouse), S824D 1st Bleed, conc: 0.1. mg/ml, PD 15/12/14
Ab: GST-Rab7A (mouse), S824D 2nd Bleed, conc: 0.14 mg/ml, PD 21/01/15
Ab: GST-Rab7A (mouse), S824D 3rd Bleed, conc: 0.13 mg/ml, PD 09/02/15



TCL = Total Cell lysate
BMDMs = mouse Bone marrow derived macrophages

Immunodetection conditions:

Blocking: 5 % milk/0.1 % TBS/T, RT, 1 h
1st Ab: 1:10 000, 5 % milk/0.1 % TBS/T, 4 °C
2nd Ab: 1: 10 000, 5 % milk/0.1 % TBS/T, 4 °C