ANTIBODY TESTING RESULTS

Standard Reporting Template

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

Date: 08/04/2015

Laboratory Name: Alessi

Your Name: Thomas Hochdörfer

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

Antibody Name: S815D SV2A phospho Thr84

Full Antigen Name: Synaptic vesicle glycoprotein 2A

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

```
>sp|Q7L0J3|SV2A_HUMAN Synaptic vesicle glycoprotein 2A OS=Homo sapiens
GN=SV2A PE=1 SV=1
MEEGFRDRAAFIRGAKDIAKEVKKHAAKKVVKGLDRVQDEYSRRSYSRFEEEDDDDDFPA
PSDGYYRGEGTQDEEEGGASSDATEGHDEDDEIYEGEYQGIPRAESGGKGERMADGAPLA
GVRGGLSDGEGPPGGRGEAQRRKEREELAQQYEAILRECGHGRFQWTLYFVLGLALMADG
VEVFVVGFVLPSAEKDMCLSDSNKGMLGLIVYLGMMVGAFLWGGLADRLGRRQCLLISLS
VNSVFAFFSSFVQGYGTFLFCRLLSGVGIGGSIPIVFSYFSEFLAQEKRGEHLSWLCMFW
MIGGVYAAAMAWAIIPHYGWSFQMGSAYQFHSWRVFVLVCAFPSVFAIGALTTQPESPRF
FLENGKHDEAWMVLKQVHDTNMRAKGHPERVFSVTHIKTIHQEDELIEIQSDTGTWYQRW
GVRALSLGGQVWGNFLSCFGPEYRRITLMMMGVWFTMSFSYYGLTVWFPDMIRHLQAVDY
ASRTKVFPGERVEHVTFNFTLENQIHRGGQYFNDKFIGLRLKSVSFEDSLFEECYFEDVT
SSNTFFRNCTFINTVFYNTDLFEYKFVNSRLINSTFLHNKEGCPLDVTGTGEGAYMVYFV
SFLGTLAVLPGNIVSALLMDKIGRLRMLAGSSVMSCVSCFFLSFGNSESAMIALLCLFGG
```

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

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: 3rd

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

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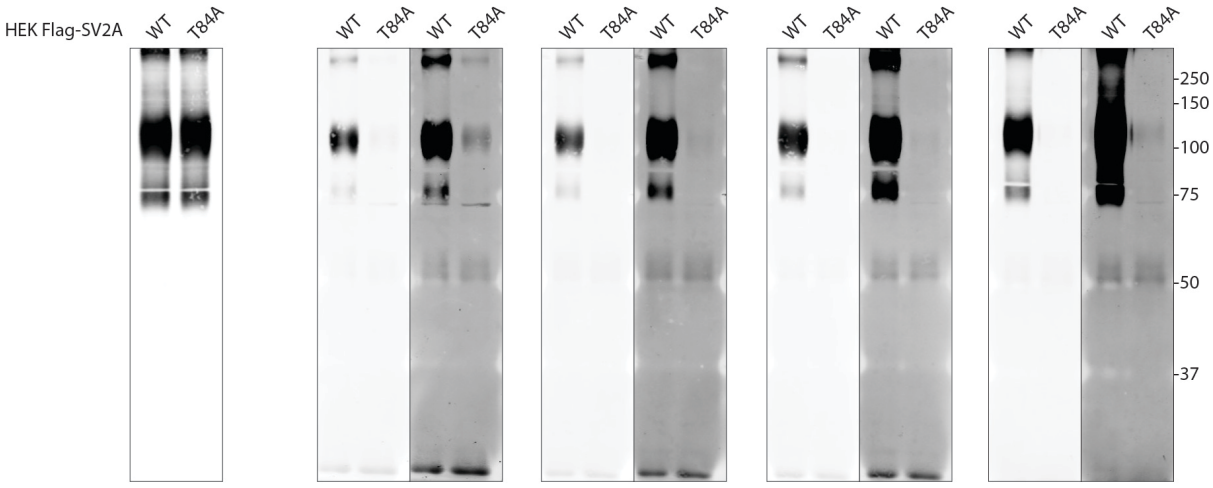
IMMUNOPRECIPITATION -- DATA



IMMUNOPRECIPITATION -- ASSOCIATED FIGURE LEGENDS

Stably transfected HEK 293 cells overexpressing FLAG-SV2A after doxycycline treatment were used, either with WT FLAG-SV2A or with the T84A mutation. 2 mg of cell lysate were subjected to FLAG-beads precipitation. 10% of lysates were loaded on SDS-PAGE and detected with all 4 bleeds. 1st bleed showed some background band in T84A cells, 3rd bleed showed the lowest background.
No whole cell lysates shown since detection with SV2A antibodies in whole cell lysates results in very dirty blots with lots of signals.

IMMUNOPRECIPITATION -- EXPERIMENTAL DESIGN

Antibody was used at 1 µg/ml in 10% milk + 0.1% Tween 20 and 10 µg/ml non-phospho peptide. Incubation over night at 4°C.

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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