

ANTIBODY TESTING RESULTS

Standard Reporting Template

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

Date:

Laboratory Name:

Your Name:

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

Antibody Name:

Full Antigen Name:

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

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MLSSRAEAAMTAADRAIQRFRLRTGAAVRYKVMKNWGVIGGIAAALAAGIYVIWGPITERK
KRRKGLVPLVNLGNTCFMNSLLQGLSACPAFIRWLEEFTSQYSRDQKEPPSHQYLSLTL
LHLLKALSCQEVTDDDEVLDASCLLDVLRMYRWQISSFEEQDAHELFHVITSSLEDERDRQ
PRVTHLFDVHSLEQQSEITPKQITCRTRGSPHPTS NHWKSQHPPFHGRLTSNMVCKHCEHQ
SPVRFDTFDSL SLSIPAATWGHPLTLDHCLHFFISSESVRDVVCNCTKIEAKGTLNGEK
VEHQRTTFVKQLKLGKLPQCLCIHLQRLSWSSHGTPLKRHEHVQFNEFLMMDIYKYHLLG
HKPSQHNPKNKPNPPTLELQDGPAPT PVLNQP GAPKTQIFMNGACSPSLLPTLSAPMP
FPLPVPDYSSSTYLFRLMAVVVHHGDMHSGHFVTYRRSPPSARNPLSTSNQWLWVSDDT
VRKASLQEVLS S SAYLLFYERVL SRMQHQS QECKSEE
    
```

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

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

- 1 2 3 4 5 6 7

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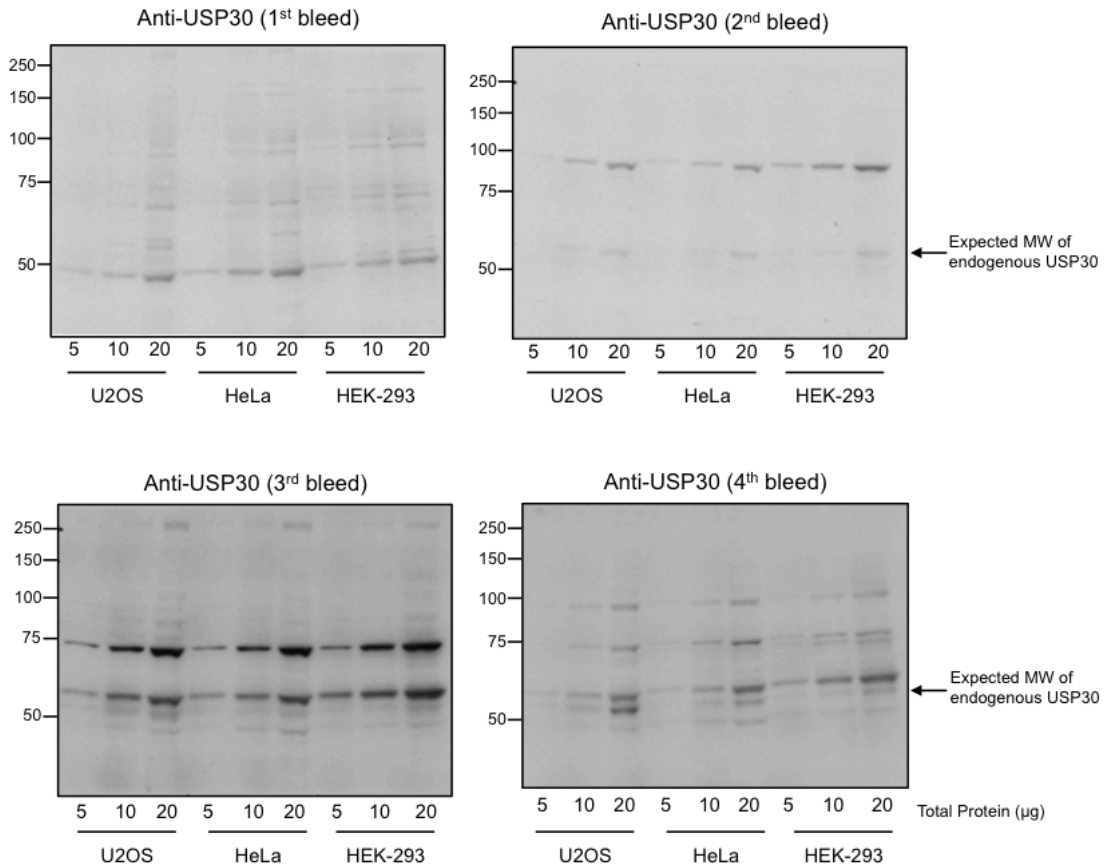
SUCCESSFUL APPLICATIONS:

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	✓	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bleed #2	✓	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bleed #3	✓	✓	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bleed #4	✓	✓	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bleed #5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bleed #6	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bleed #7	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

BEST Working Bleed: 3rd and 4th bleeds

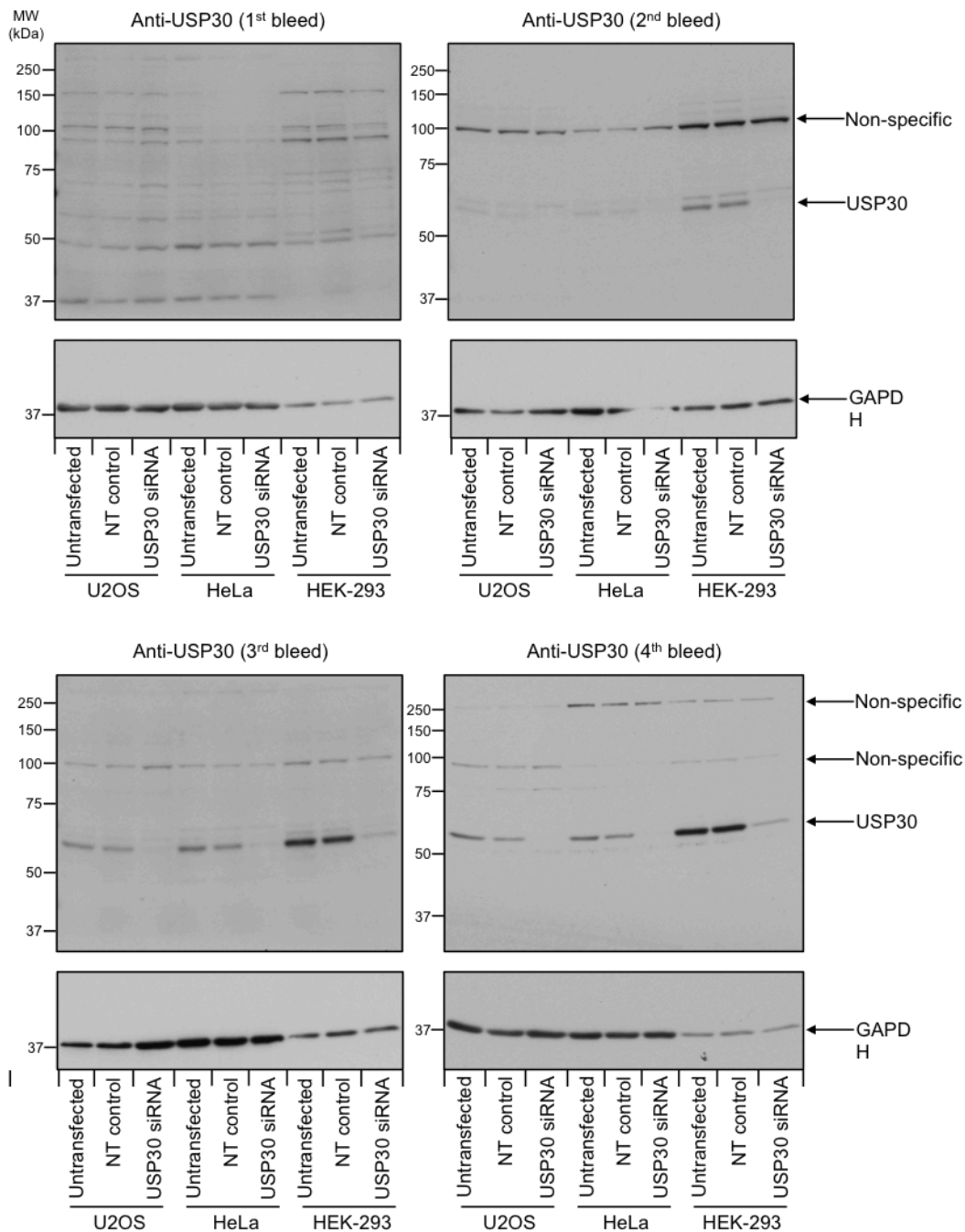
Immunoblotting results: (Luke Hutchinson)



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1. Immunodetection of endogenous USP30. All four bleeds of the anti-USP30 antibody were tested for the immunodetection of endogenous USP30 in three different human cell lines (U2OS osteosarcoma cells, HeLa adenocarcinoma cells and HEK-293 embryonic kidney cells). Antibody bleeds 1 and 2 exhibit non-specific binding and do not appear to detect USP30. By contrast, antibody bleeds 3 and 4 appear to detect a band at the appropriate size (USP30 MW = 58.5 kDa) although they also detect non-specific proteins at approximately 75 kDa and 100 kDa.



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2. Validation of anti-USP30 antibody bleeds using siRNA-mediated depletion of USP30 protein level. Three different human cell lines, U2OS osteosarcoma cells, HeLa adenocarcinoma cells and HEK-293 embryonic kidney cells were transfected with either non-targeting siRNA or USP30 siRNA for 48-hours at a concentration of 40 nM. Antibody bleeds 1 and 2 exhibit non-specific binding, whereas bleeds 3 and 4 detect a prominent band at approximately 58 kDa that is reduced in USP30 siRNA samples.

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*)

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

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

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

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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