DATA NOTE

Identification of highly specific antibodies for Serine/threonine-protein kinase TBK1 for use in immunoblot, immunoprecipitation and immunofluorescence [version 1; peer review: awaiting peer review]

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First published: 24 Aug 2022, 11:977
https://doi.org/10.12688/f1000research.124632.1
Latest published: 24 Aug 2022, 11:977
https://doi.org/10.12688/f1000research.124632.1

Abstract
TBK1 is a serine-threonine protein kinase that has been linked to a number of diseases including amyotrophic lateral sclerosis and frontotemporal dementia. Reproducible research on TBK1 has been hampered by the lack of well characterized antibodies. In this study, we characterized 11 commercial antibodies for TBK1 for use in immunoblot, immunofluorescence and immunoprecipitation, using an isogeneic knock-out cell line as a control. We identify antibodies that appear specific for all three applications but invite the readers to interpret the present findings based on their own scientific expertise and use this report as a guide to select the most appropriate antibody for their specific needs.

Keywords
TBK1, Uniprot# Q9UHD2, antibody characterization, antibody validation, Western blot, immunoblot, immunoprecipitation, immunofluorescence
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Author roles: Alshafie W: Investigation, Methodology; Fotouhi M: Investigation; Shlaifer I: Investigation; Ayoubi R: Visualization, Writing – Original Draft Preparation; Edwards AM: Conceptualization, Funding Acquisition, Writing – Original Draft Preparation; Durcan TM: Conceptualization, Funding Acquisition, Resources; McPherson PS: Conceptualization, Funding Acquisition, Resources, Supervision, Writing – Original Draft Preparation; Laflamme C: Conceptualization, Data Curation, Funding Acquisition, Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: The laboratory of Peter S McPherson was awarded a Genomic Applications Partnership Program grant from Genome Canada in 2021. For this project, the laboratory of Peter McPherson developed partnerships with high-quality antibody manufacturers and knockout cell lines providers. The partners provide antibodies and knockout lines to the McPherson laboratory at no cost. Partners are: -Abcam -Abclonal -Aviva Systems Biology -Bio-Techne -Cell Signaling Technology -Developmental Studies Hybridoma Bank -Genetex -Horizon Discovery -Proteintech -Synaptic Systems -Thermo Fisher Scientific

Grant information: This work was supported by a grant from the Motor Neurone Disease Association (UK), The ALS Association (USA) and ALS Canada, by a Canadian Institutes of Health Research Foundation Grant (FDN154305) and by the Government of Canada through Genome Canada and Ontario Genomics (OGI-210). The Structural Genomics Consortium is a registered charity (no. 1097737) that receives funds from Bayer AG, Boehringer Ingelheim, Bristol Myers Squibb, Genentech, Genome Canada through Ontario Genomics Institute (grant no. OGI-196), the EU and EFPIA through the Innovative Medicines Initiative 2 Joint Undertaking (EUBOPEN grant no. 875510), Janssen, Merck KGaA (also known as EMD in Canada and the United States), Pfizer and Takeda. WA is supported by a Mitacs postdoctoral fellowship.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Alshafie W, Fotouhi M, Shlaifer I et al. Identification of highly specific antibodies for Serine/threonine-protein kinase TBK1 for use in immunoblot, immunoprecipitation and immunofluorescence [version 1; peer review: awaiting peer review] F1000Research 2022, 11:977 https://doi.org/10.12688/f1000research.124632.1
**Introduction**

The lack of robust characterization for research antibodies contributes to the reproducibility crisis.\(^1\) Given that there are more than five million antibodies on the commercial market (CiteAb.com), we hypothesize that with appropriate characterization criteria and testing, we should be able to identify high performing antibodies for many if not most proteins in the human genome.\(^2\)

TBK1 regulates autophagy through phosphorylation of Optineurin\(^3\) and the C9ORF72/SMCR8 complex.\(^4\) Of note, mutations in both Optineurin\(^5\) and the C9ORF72/SMCR8 complex\(^6,7\) cause monogenic forms of amyotrophic lateral sclerosis and frontotemporal dementia. Moreover, TBK1 also phosphorylates LC3C, GABARAP-L2\(^8\) and AKT1\(^9\) promoting autophagy.

The endogenous localization of TBK1 under the basal state and during autophagy remains to be determined. Moreover, TBK1 protein interactomes have been determined using overexpression systems, with the exception of one study.\(^10\) TBK1 antibodies are key to address these unknowns.

To explore the availability of high-quality antibodies for human proteins, we devised an antibody characterization strategy in which we use wild-type (WT) and isogenic knockout (KO) control cells to perform head-to-head comparisons of all available commercial antibodies in immunoblot (Western blot), immunoprecipitation and immunofluorescence applications.\(^11\) Here, we apply this approach to TBK1 and identify specific antibodies for all tested applications, enabling biochemical and cellular assessment of TBK1.

**Validation and discussion**

To identify a cell line that expresses adequate levels of TBK1 protein to provide sufficient signal to noise, we examined the DepMap public proteomic database (depmap.org, RRID:SCR_017655). U2OS was selected as the expression of TBK1 protein level is in the average range of cancer cells analyzed,\(^12\) is easily amenable to CRISPR-Cas9 and is a rather flat cell line ideal for immunofluorescence studies. U2OS was modified with CRISPR/Cas9 to knockout the corresponding TBK1 gene (Table 1).\(^13\)

Extracts from wild-type and TBK1 KO cells were prepared and used to probe 11 commercial antibodies from 6 companies (Table 2) by immunoblot (Western blot) and immunoprecipitation. The profile of each of the antibodies is shown in Figures 1, 2 and 3.

Antibodies were screened by immunofluorescence using a mosaic strategy.\(^11\) WT cells were labelled with a green fluorescent dye, whereas the KO cells were labelled with a far-red fluorescent dye. A third channel was used to image the primary antibodies. Plating WT and KO cells together and imaging both cell type in the same field of view reduces imaging and analysis biases.

In conclusion, we have screened TBK1 commercial antibodies by immunoblot, immunoprecipitation and immunofluorescence. The data provided can be used as a guide to purchase the most appropriate antibody for a researcher’s needs.

**Methods**

**Antibodies**

All TBK1 antibodies are listed in Table 2. Peroxidase-conjugated goat anti-mouse and anti-rabbit antibodies are from Thermo Fisher Scientific (cat. number 65-6120 and 62-6520). Alexa-555-conjugated goat anti-mouse and anti-rabbit secondary antibodies are from Thermo Fisher Scientific (cat. number A21424 and A21429).

**CRISPR/Cas9 genome editing**

Cell lines used are listed in Table 1. U2OS TBK1 KO clone was generated using an open-access protocol\(^13\) with an inducible Cas9 U2OS line.\(^11\) Two guide RNAs (purchased at Synthego) were used to introduce a STOP codon in the

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**Table 1. Summary of the cell lines used.**

<table>
<thead>
<tr>
<th>Institution</th>
<th>RRID (Cellosaurus)</th>
<th>Cell line</th>
<th>genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montreal Neurological Institute</td>
<td>CVCL_0042</td>
<td>U2OS</td>
<td>WT</td>
</tr>
<tr>
<td>Montreal Neurological Institute</td>
<td>CVCL_A6LQ</td>
<td>U2OS</td>
<td>TBK1 KO</td>
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</table>
Table 2. Summary of the Serine/threonine-protein kinase TBK1 antibodies tested.

<table>
<thead>
<tr>
<th>Company</th>
<th>Catalog number</th>
<th>Lot number</th>
<th>RRID (Antibody Registry)</th>
<th>Clonality</th>
<th>Clone ID</th>
<th>Host</th>
<th>Concentration (μg/μl)</th>
<th>Vendors recommended applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-Techne</td>
<td>NB100-56705</td>
<td>B-1</td>
<td>AB_838420</td>
<td>monoclonal</td>
<td>108A429</td>
<td>mouse</td>
<td>1.00</td>
<td>Wb, IF</td>
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<tr>
<td>Proteintech</td>
<td>28397-1-AP</td>
<td>00076443</td>
<td>AB_2881132</td>
<td>polyclonal</td>
<td>-</td>
<td>rabbit</td>
<td>0.43</td>
<td>Wb</td>
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<tr>
<td>Proteintech</td>
<td>67211-1-Ig</td>
<td>10013180</td>
<td>AB_2882504</td>
<td>monoclonal</td>
<td>2D7B1</td>
<td>mouse</td>
<td>1.00</td>
<td>Wb, IF</td>
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<td>Thermo Fisher Scientific</td>
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<td>VL3152289A</td>
<td>AB_10981817</td>
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<td>-</td>
<td>rabbit</td>
<td>not provided</td>
<td>Wb, IF, IP</td>
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<tr>
<td>Thermo Fisher Scientific</td>
<td>703154</td>
<td>2274494</td>
<td>AB_2848223</td>
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<td>JM42-11</td>
<td>rabbit</td>
<td>0.50</td>
<td>Wb, IF</td>
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<td>ab12116</td>
<td>GR3334526-1</td>
<td>AB_298856</td>
<td>monoclonal</td>
<td>108A429</td>
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<td>EPR2867 (2)-19</td>
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<td>43481</td>
<td>AB_11174793</td>
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<td>-</td>
<td>rabbit</td>
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<td>Wb, IF</td>
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<td>38066</td>
<td>1</td>
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<td>E8I3G</td>
<td>rabbit</td>
<td>not provided</td>
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<td>-</td>
<td>AB_2255663</td>
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<td>D1B4</td>
<td>rabbit</td>
<td>not provided</td>
<td>Wb, IP</td>
</tr>
</tbody>
</table>

Wb=Western blot; IF=immunofluorescence; IP=immunoprecipitation.
**Cell culture**

Cells were cultured in DMEM high-glucose (GE Healthcare cat. number SH30081.01) containing 10% fetal bovine serum (Wisent, cat. number 080450), 2 mM L-glutamate (Wisent cat. number 609065), 100 IU penicillin and 100 μg/ml streptomycin (Wisent cat. number 450201).

**Figure 1. Serine/threonine-protein kinase TBK1 antibody screening by immunoblot.** Lysates of U2OS (WT and TBK1 KO) were prepared and 50 μg of protein were processed for immunoblot with the indicated TBK1 antibodies. The Ponceau stained transfers of each blot are presented to show equal loading of WT and KO lysates and protein transfer efficiency from the acrylamide gels to the nitrocellulose membrane. Antibody dilution used was 1/5000 for all tested antibodies. Predicted band size: ~83 kDa.

*TBK1* gene (sequence guide 1: UUUGAACAUCCACUGGACGA, sequence guide 2: CAAAUUAUUUGCUAUUGAAG).
Antibody screening by immunoblot

Immunoblots were performed as described in our standard operating procedure.\textsuperscript{14} Lysates were sonicated briefly and incubated 30 min on ice. Lysates were spun at ~110,000 \( \times \) g for 15 min at 4°C and equal protein aliquots of the supernatants were analyzed by SDS-PAGE and immunoblot. BLUelf prestained protein ladder from GeneDireX (cat. number PM008-0500) was used.

Immunoblots were performed with large 5-16% gradient polyacrylamide gels and transferred on nitrocellulose membranes. Proteins on the blots were visualized with Ponceau staining which is scanned at 300 dpi using a regular flatbed scanner to show together with individual immunoblot. Blots were blocked with 5% milk for 1 hr, and antibodies were incubated O/N at 4°C with 5% bovine serum albumin in TBS with 0.1% Tween 20 (TBST). Following three washes with TBST, the peroxidase conjugated secondary antibody was incubated at a dilution of ~0.2 \( \mu \)g/ml in TBST with 5% milk for 1 hr at room temperature followed by three washes with TBST. Membranes are incubated with ECL from Pierce (cat. number 32106) prior to detection with HyBlot CL autoradiography films from Denville (cat. number 1159T41).

Antibody screening by immunoprecipitation

Immunoprecipitation was performed as described in our standard operating procedure.\textsuperscript{15} Antibody-bead conjugates were prepared by adding 1.0 \( \mu \)g of antibody to 500 ul of PBS with 0.01% triton X-100 in a microcentrifuge tube, together with 30 \( \mu \)l of protein A- (for rabbit antibodies) or protein G- (for mouse antibodies) Sepharose beads. Tubes were rocked O/N at 4°C followed by several washes to remove unbound antibodies.

U2OS WT were collected in HEPES buffer (20 mM HEPES, 100 mM sodium chloride, 1 mM EDTA, 1% Triton X-100, pH 7.4) supplemented with protease inhibitor. Lysates are rocked 30 min at 4°C and spun at 110,000xg for 15 min at 4°C. One ml aliquots at 1.0 mg/ml of lysate were incubated with an antibody-bead conjugate for ~2 hrs at 4°C. Following centrifugation, the unbound fractions were collected, and beads were subsequently washed three times with 1.0 ml of HEPES lysis buffer and processed for SDS-PAGE and immunoblot on a 5-16% acrylamide gel.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure.\textsuperscript{16} U2OS WT and \( \text{TBK1} \) KO were labelled with a green and a deep red fluorescence dye, respectively. The fluorescent dyes used are from Thermo Fisher Scientific.
Scientific (cat. number C2925 and C34565). WT and KO cells were plated on glass coverslips as a mosaic and incubated for 24 hrs in a cell culture incubator. Cells were fixed in 4% PFA (in PBS) for 15 min at room temperature and then washed 3 times with PBS. Cells were permeabilized in PBS with 0.1% Triton X-100 for 10 min at room temperature and blocked with PBS with 5% BSA, 5% goat serum and 0.01% Triton X-100 for 30 min at room temperature. Cells were incubated with IF buffer (PBS, 5% BSA, 0.01% Triton X-100) containing the primary TBK1 antibodies O/N at 4°C. Cells were then washed 3 x 10 min with IF buffer and incubated with corresponding Alexa Fluor 555-conjugated secondary antibodies in IF buffer at a dilution of 1.0 μg/ml for 1 hr at room temperature with DAPI. Cells were washed 3 x 10 min with IF buffer and once with PBS. Coverslips were mounted on a microscopic slide using fluorescence mounting media (DAKO). Imaging was performed using a Zeiss LSM 880 laser scanning confocal microscope equipped with a Plan-Apo 40x oil objective (NA = 1.40). Resulting images were cropped and adjusted for brightness and contrast using the Zen navigation software (Zeiss, Zen blue 3.4.91.00000). All cell images represent a single focal plane. Figures were assembled with Adobe Illustrator (version 26.3.1).

**Data availability**

**Underlying data**


Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Acknowledgments

We thank Chetan Raina (YCharOS Inc.) for his important contribution to the creation of an open scientific ecosystem of antibody manufacturers and knockout cell line suppliers.

A previous version of this article was published on bioRxiv: https://doi.org/10.1101/2022.06.03.494699.

References

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