The Michael J. Fox Foundation's Strategy to Generate, Characterize, and Distribute Preclinical Antibody Tools for Investigating Parkin/PINK1 and LRRK2- or PINK1-Related Rab Molecular Biology

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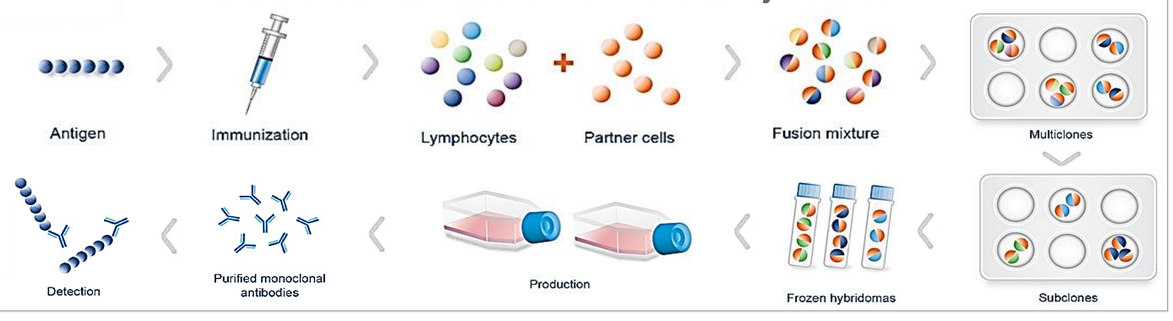
Introduction

A field-wide challenge in Parkinson's disease (PD) research is a general lack of availability for high-quality, reproducible, and readily accessible preclinical research tools. To address these challenges, The Michael J. Fox Foundation for Parkinson's Research (MJFF) has developed a growing resource of preclinical tools for the PD research and drug development communities that endeavors to provide researchers with easy access to rigorously validated, research-enabling preclinical tools for molecular biology studies. An important aspect of MJFF's preclinical tools portfolio are monoclonal antibodies that target PD-relevant proteins. In collaboration with academic experts and in partnership with Abcam and BioLegend, MJFF has sponsored the custom generation and independent validation of several monoclonal antibodies targeting both total and phosphorylated or modified versions of PD-relevant proteins including Parkin, PINK1, and LRRK2- and PINK1-related Rab proteins. Parkin and PINK1 (PTEN-induced putative kinase 1) are implicated in mitochondrial homeostasis pathways. Bi-allelic mutations in Parkin and PINK1 genes underlie young-onset, autosomal recessive PD. The Rab superfamily of proteins function generally in endocytosis, and a subset of Rab family members have been identified as key phosphorylation substrates of LRRK2 and PINK1 kinase activity, respectively. A select number of bona fide mutations in the gene LRRK2, which encodes the LRRK2 (leucine-rich repeat kinase 2) protein, are linked to late-onset, autosomal dominant PD, and these mutants increase LRRK2's kinase activity. Herein we discuss the general MJFF antibody generation strategy and provide characterization data for ongoing custom antibody development projects, as well as antibody pipeline updates and commercial launch timelines for MJFF's cumulative antibody collection. Ultimately, these MJFF-sponsored antibody projects aim to address field-wide challenges in the PD preclinical tools and reagents landscape and to overall accelerate Parkinson's disease research.

MJFF-Abcam Rabbit Monoclonal Antibody Pipeline

Antibody	Antibody Descrption	Stage of Development	Est. Availability
MJF17 (pS65 Parkin)	Recombinant rabbit monoclonal anti-human pS65 Parkin	QC for Distribution	Early 2018
MJF18 (pT257 PINK1)	Rabbit monoclonal anti-human pT257 PINK1	QC for Distribution	Early 2018
MJF19 (pS1292 LRRK2)	Recombinant rabbit monoclonal anti-human pS1292 LRRK2	Available	Available
MJF20 (pT72 Rab8)	Recombinant rabbit monoclonal anti-human pT72 Rab8a/b	Purified Recombinant	Early 2018
MJF21 (pT73 Rab10)	Recombinant rabbit monoclonal anti-human pT73 Rab10	Purified Recombinant	Early 2018
MJF22 (total Rab8)	Recombinant rabbit monoclonal anti-human total Rab8a	Subclones	Mid 2018
MJF23 (total Rab10)	Recombinant rabbit monoclonal anti-human total Rab10	Fusion	Late 2018
MJF24 (pS106 Rab12)	Recombinant rabbit monoclonal anti-human pS106 Rab12	Fusion	Late 2018
MJF25 (pT71 Rab29)	Recombinant rabbit monoclonal anti-human pT71 Rab29 (Rab7L1)	Fusion	Late 2018
MJF26 (pS65 Ubiquitin)	Recombinant rabbit monoclonal anti-human pS65 Ubiquitin	Immunization	Early 2019
MJF27 (pS111 Rab8)	Recombinant rabbit monoclonal anti-human pS111 Rab8	Immunization	Early 2019
MJF28 (pS1292 LRRK2)	Recombinant rabbit monoclonal anti-human pS1292 LRRK2	Immunization	Early 2019
MJF29 (pT1357 LRRK2)	Recombinant rabbit monoclonal anti-human pT1357 LRRK2	Antigen Design	Mid 2019
MJF30 (total Rab29)	Recombinant rabbit monoclonal anti-human total Rab29 (Rab7L1)	Antigen Design	Mid 2019
MJF31 (total Rab12)	Recombinant rabbit monoclonal anti-human total Rab12	Antigen Design	Mid 2019
MJF32 (total PINK1)	Recombinant rabbit monoclonal anti-human total PINK1	Antigen Design	Mid 2019

Abcam Recombinant Rabbit Monoclonal Antibody Production



MJFF Parkin Antibodies with BioLegend and OHRI

Antibody	Antibody Description	Stage of Development
FL human Parkin	Mouse monoclonal anti-human Parkin	Characterization in progress
Dopamine-modified human Parkin	Mouse monoclonal anti-DA-modified human Parkin *(LaVoie et al. Nat Med 2005)*	Immunization Q4 2017

Anti-human Parkin Monoclonal Antibodies: Screening

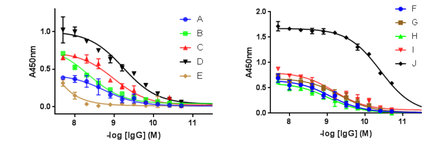
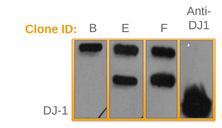


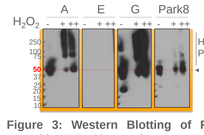
Figure 1: Binding curves for newly developed monoclonal antibodies to full-length, human Parkin. A to E (left) and F to J (right) against plate-immobilized human recombinant full-length Parkin protein. The binding curves confirmed that the majority of clones have avid binding to human Parkin with EC50's of ~0.05 to 1.0 nM.



Summary

	A	B	C	D	E	F	G	H	I	J
FL	+	+	+	+	+	+	+	+	+	+
321C	+	-	+	+	+	+	+	+	+	+

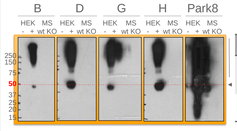
Figure 2: Slot Blotting of Recombinant, Untagged, Human PD-Linked Proteins. 5 µg of recombinant Parkin proteins and 20 µg DJ-1 were loaded onto a nitrocellulose filter membrane under non-denaturing conditions. mAbs were used at 1:1K; except for E (1:500) and A (1:5K).



Summary

	A	B	C	D	E	F	G	H	I	J
FL	+	+	+	+	-	+	+	+	+	+
321C	+	-	+	+	-	+	+	+	+	+

Figure 3: Western Blotting of Recombinant, Full Length Parkin. 50 ng of recombinant Parkin protein was treated with 0, 50 and 500 nM H_2O_2 for 30 min at 37°C in the absence of reducing agent, then run under denaturing conditions. Abs: 1:1K; exception E (1:500), A (1:5K), Park8 (1:20K). Note the detection of monomeric (50 kDa) and oligomeric, HMW forms of Parkin (described by LaVoie *et al.* J. Neurochem 2007).



Summary

	A	B	C	D	E	F	G	H	I	J
Cellular hParkin	+	+	+	+	-	+	+	+	+	+
Murine parkin	-	-	-	-	-	-	-	-	-	-

Figure 4. Western Blotting of Cellular, Ectopic Wild-Type Parkin and Mouse Brain. 15 µg of HEK293 cell lysates overexpressing Flag Parkin (+) or Flag vector control (-) and 20 µg of WT and *park2* knock-out (KO) mouse (MS) brain lysates are shown. Lysates were run under SDS/PAGE conditions in the presence of a reducing agent. Ab concentrations: all at 1:1K; with the exception of Park8 (1:20K) and clone A (1:5K).

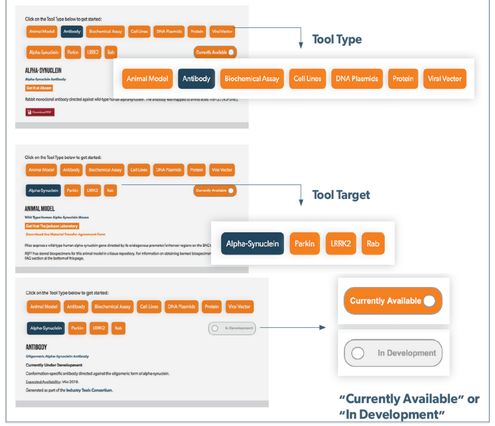
MJFF's Antibody Validation and Characterization Strategy

MJFF invests in the development and distribution of research tools to accelerate PD-related research and therapeutic development. In collaboration with its research partners and independent field experts, every MJFF-generated antibody is rigorously validated and characterized, deploying the following criteria:

- Thorough specificity and selectivity analyses
- Candidate antibodies are screened in physiologically relevant conditions
- Antibody candidates are validated against knockout or de-phospho or non-mutant controls
- During clone selection, antibodies are screened in multiple applications, including immunoblot, immunoprecipitation, immunocyto- or immunohistochemistry, and ELISA
- All MJFF antibodies are epitope mapped

MJFF Preclinical Tools Resources

All MJFF-generated antibodies are listed on the MJFF Online Research Tools Catalog. To access a full list of molecular tools and preclinical research models generated and validated by MJFF, visit www.michaeljfox.org/toolscatalog.



Summary and More Information

MJFF is invested in providing the PD research community with high-quality tools and models to support rapid new discoveries and encourage reliable, reproducible data. The tools described in this poster are the result of recent collaborative efforts aimed at generating antibodies in particular.

Information on other MJFF preclinical tools for additional PD-related targets can be found in the Research Tools Catalog at www.michaeljfox.org/toolscatalog. Questions regarding MJFF research tools can be sent to tools@michaeljfox.org.