What do you really know about that antibody?

Maryann E. Martone, Ph. D.
FAIR Data Informatics Lab
University of California, San Diego
SciCrunch Inc

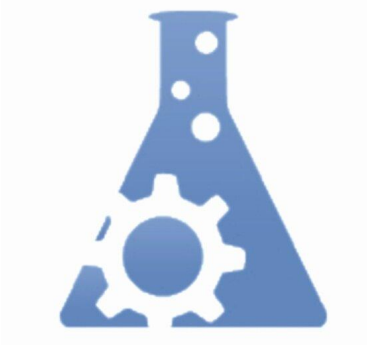
An NIDDK Resource

Ask dknet.org

The NIDDK Information Network

Disclosures

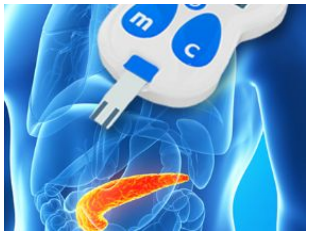
Maryann Martone is a founder and CSO of SciCrunch Inc, a start up building tools and services around Research Resource Identifiers (RRIDs)



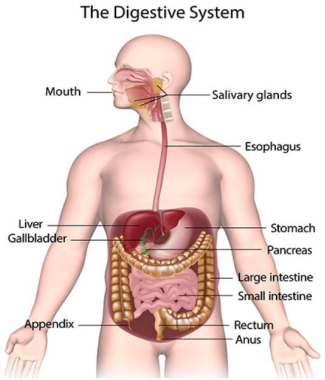
https://www.scicrunch.com/

WHO IS dkNET'S TARGET AUDIENCE?

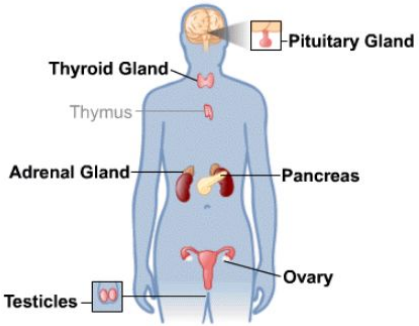
- Basic and clinical researchers in diabetes, digestive, obesity, endocrine, metabolic, kidney, urologic, nutrition, bone and blood diseases.
- Tools and services are **relevant across all biomedical domains**



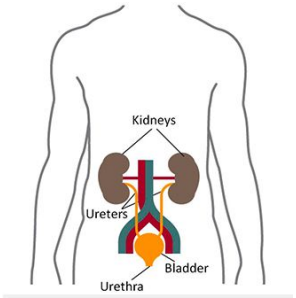
Diabetes



Digestive System



Endocrine System



Urinary System

Image credits: NIDDK, NLM

About dkNET

- Research resource information portal for biomedical researchers
- Information network to connect DK researchers and NIH-funded centers
- Funded by National Institute of Health (NIH) - National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
- Developed and maintained by the FAIR Data Informatics Laboratory (fdilab.org) at UCSD (Supports major informatics projects in neuroscience and biomedicine)

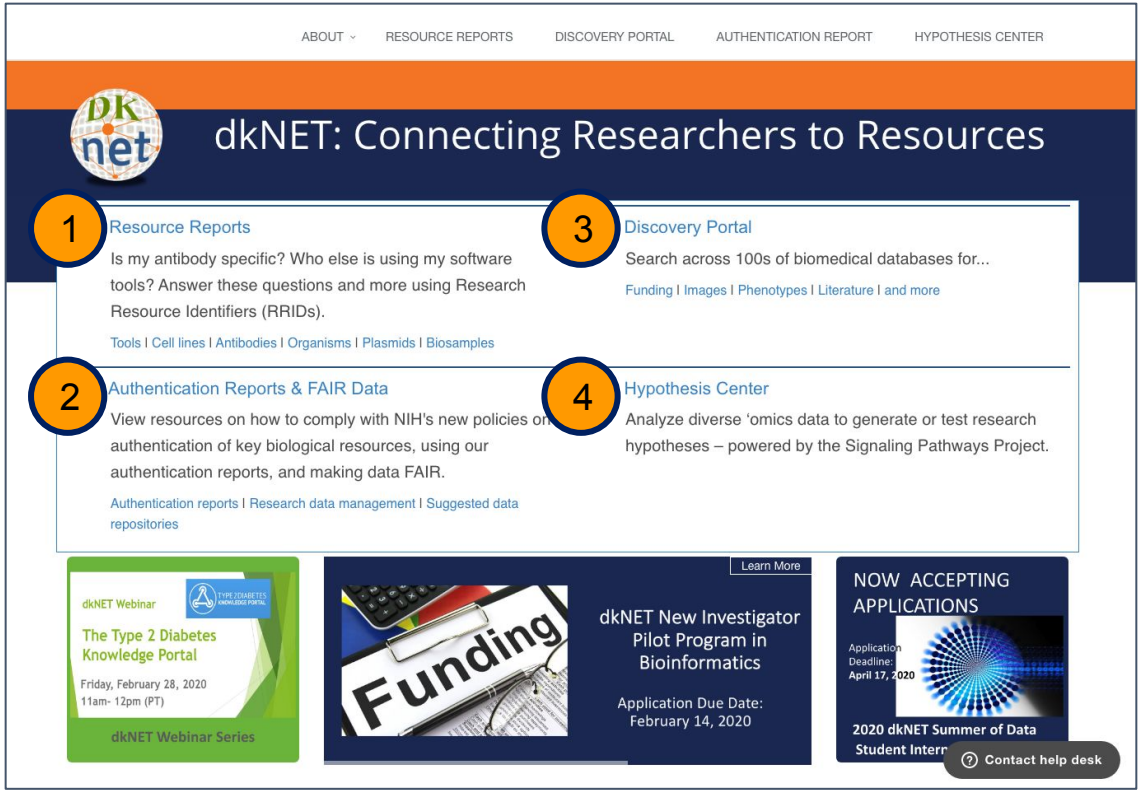
FDI Lab
FAIR Data Informatics

NIH National Institute of Diabetes and Digestive and Kidney Diseases

dkNET Portal https://dknet.org

Use dkNET to:

1. Find and evaluate research resources for your research
2. Comply with funder mandates on rigor and reproducibility and FAIR data
3. Search across 100's of biomedical databases
4. Access powerful bioinformatics tools

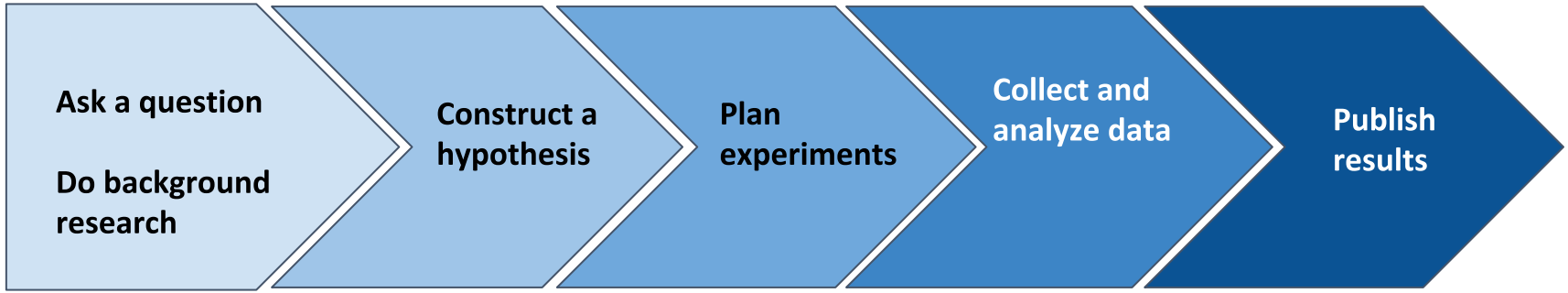


Hypothesis Center - Signaling Pathways Project

SPP simplifies data mining of ‘omics data, connects bench researchers to FAIR data to allow them to easily interrogate the data to generate hypotheses

- **Find genes** with important roles in receptors, enzymes, organs and tissues,
- **Define signaling pathways** relevant to a single gene or a regulation,

How Can dkNET Help Researchers?



Discovery Portal
- Information
- Material
- Data
- Tools
- Funded grant and funding opportunities
- Literature
- Tutorials

Hypothesis Center

Resource Reports

Authentication Reports
- Resource Identification
- Authentication plans
- NIH Mandates on Rigor and Reproducibility for grant submission

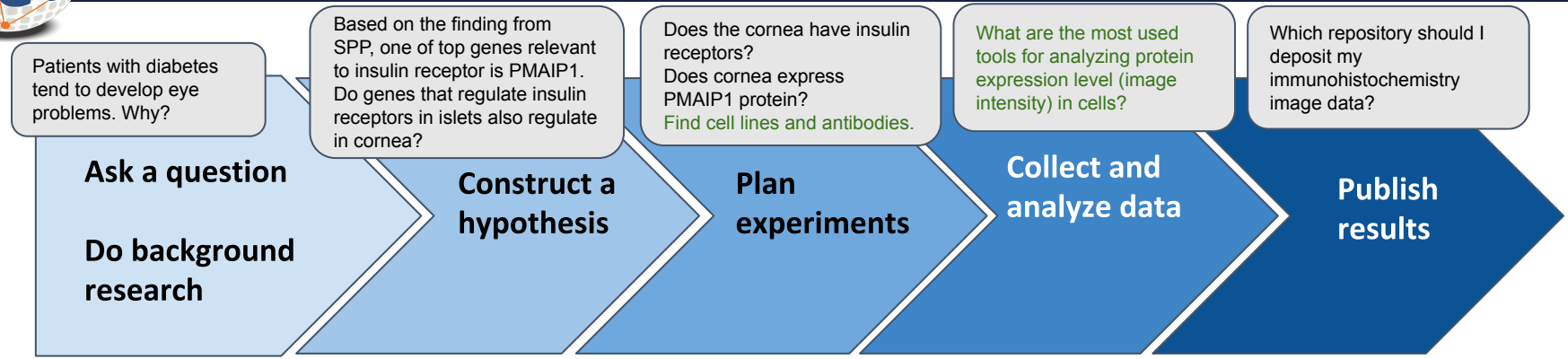
Resource Reports

Hypothesis Center

FAIR Data Resources
- Data Management
- Data Repositories

Resource Reports
- Cite RRID
- Track Resources

How Can dkNET Help Researchers? (DEMO)



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Resource Reports

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Resource Reports

Hypothesis Center

FAIR Data Resources
- Data Management
- Data Repositories

Resource Reports
- Cite RRID
- Track Resources

What is a research resource?

Research resources are any tools used by researchers in the conduct of their studies. Examples include reagents, digital tools, DNA constructs, cells, samples and organisms.

And what do you know about them?

Incorrect ICBM-DTI-81 atlas orien and white matter labels

T. Rohlfing*

RESEARCH ARTICLE

The Effects of FreeSurfer Version, Workstation Type, and Macintosh Operating System Version on Anatomical Volume and Cortical Thickness Measurements

1. How do I decide which disease model is best for me? What is the best model for my disease of interest? What is your most

rch the literature thoroughly, talk to colleagues, and review strain datasheets. The g the specific strain curated into a reference list. The most popular strain is not given research question.

Science and Society | Published: 07 May 2010

Cell line misidentification: the beginning of the end

RESEARCH ARTICLE

The ghosts of HeLa: How cell line misidentification contaminates the scientific literature

Serge P. J. M. Horbach, Willem Halffman

Published: October 12, 2017 • https://doi.org/10.1371/journal.pone.0186281

Investigation of Cross-Contamination and Misidentification of 278 Widely Used Tumor Cell Lines

Yaqing Huang, Yuehong Liu, Congyi Zheng, Chao Shen

Published: January 20, 2017 • https://doi.org/10.1371/journal.pone.0170384

Science News *from research organizations*

Misidentified and contaminated cell lines lead to faulty cancer science, experts say

Date: June 21, 2012

Source: University of Colorado Denver

Summary: Due to a high rate of contamination, misidentification and redundancy in widely available cell lines, researchers may be drawing faulty conclusions, experts say.

Cautionary notes on the use of NF-κB p65 and p5 antibodies for CNS studies

Miles Herkenham, Priyanka Rathore, Pierre Brown and Samuel J Listwak

Journal of Neuroinflammation 2011 **8**:141

https://doi.org/10.1186/1742-2094-8-141 | © Herkenham et al; licensee BioMed Central Ltd. 2011

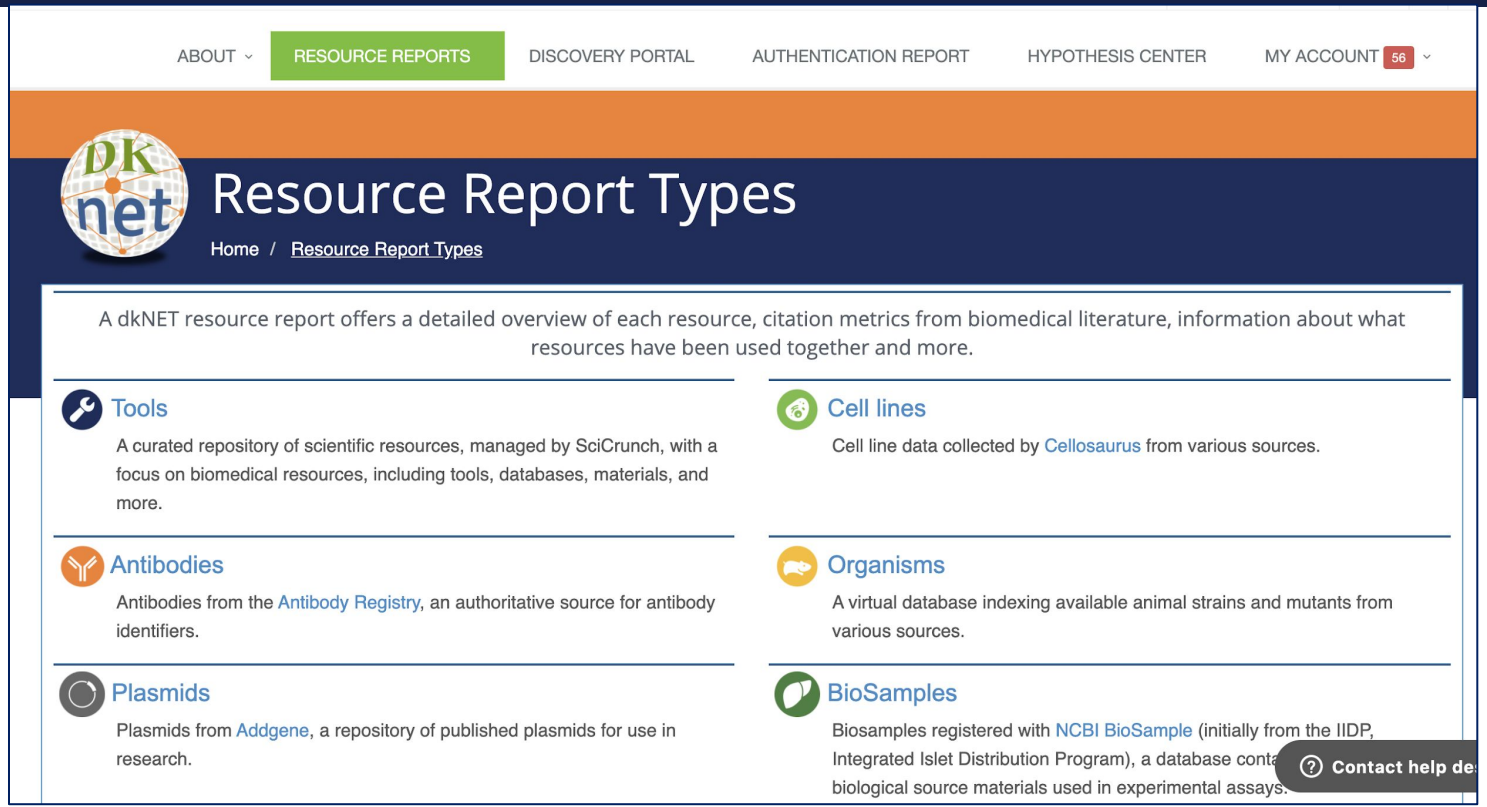
Resource Reports

Find and evaluate key research resources:

Antibodies, organisms, cell lines, tools and services, plasmids, biosamples

What resources can I use…?

dkNET provides unified search of key registries for research resources

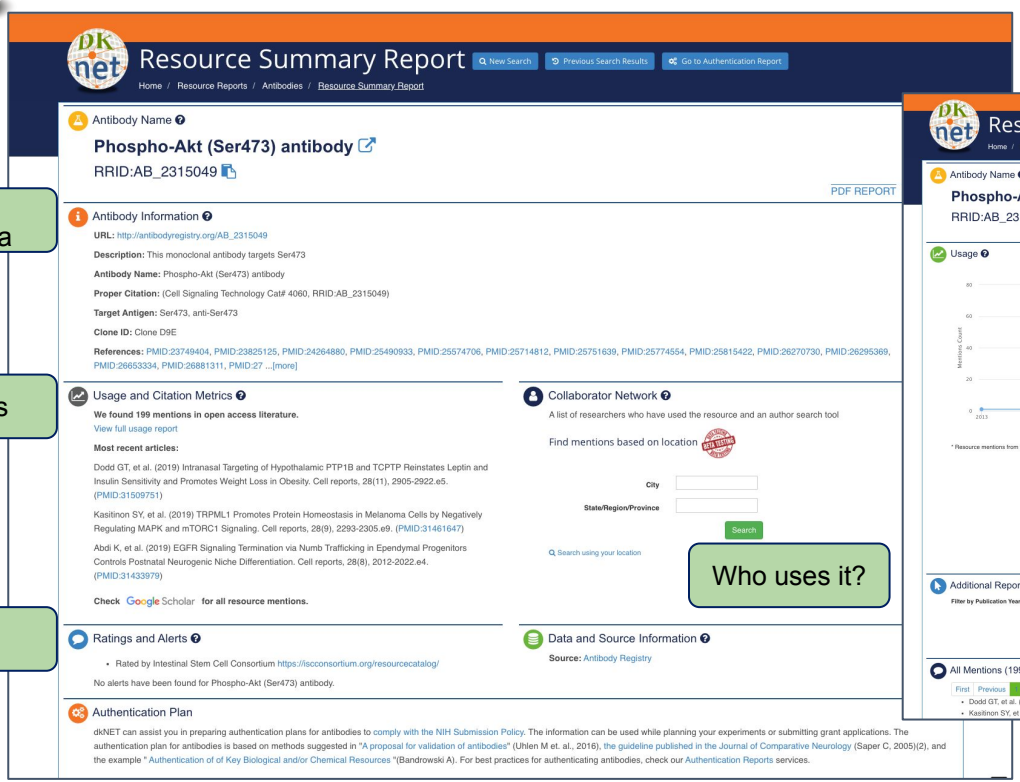


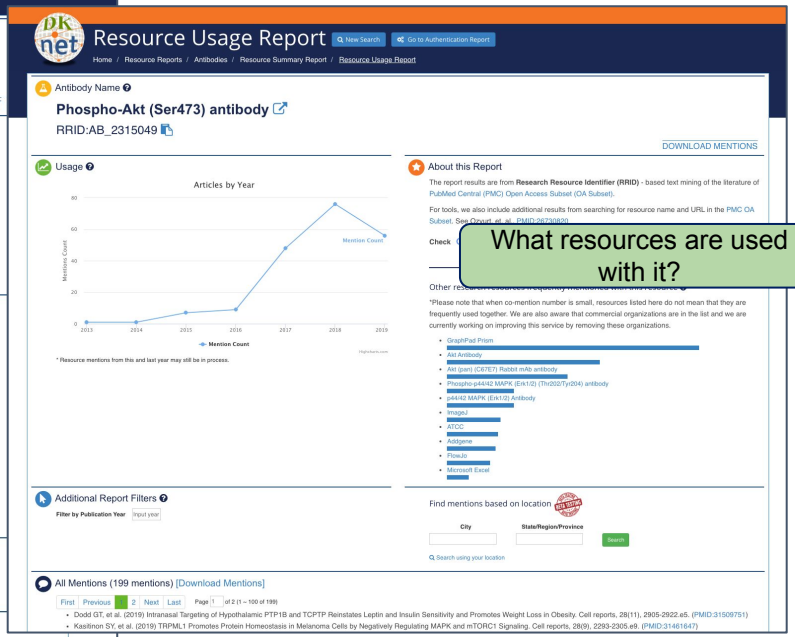
Resource Reports

Key metadata

Citations

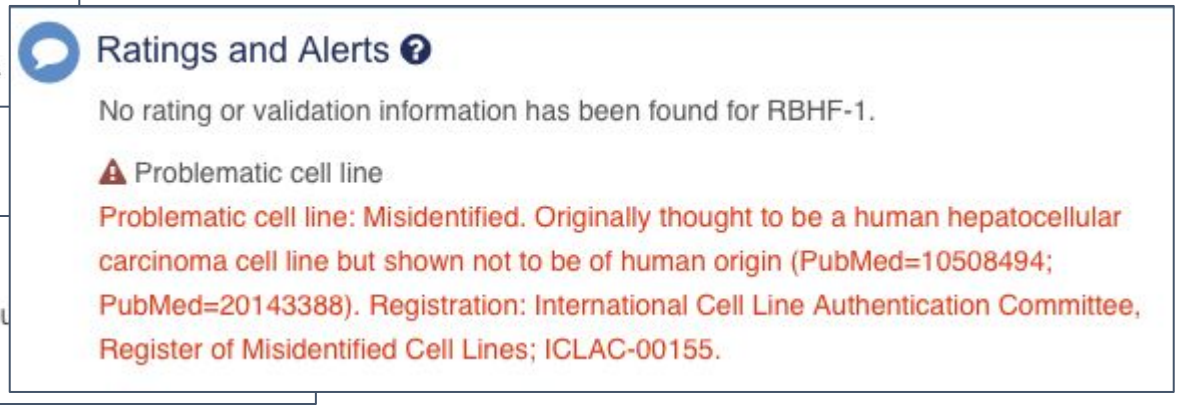
Alerts ratings





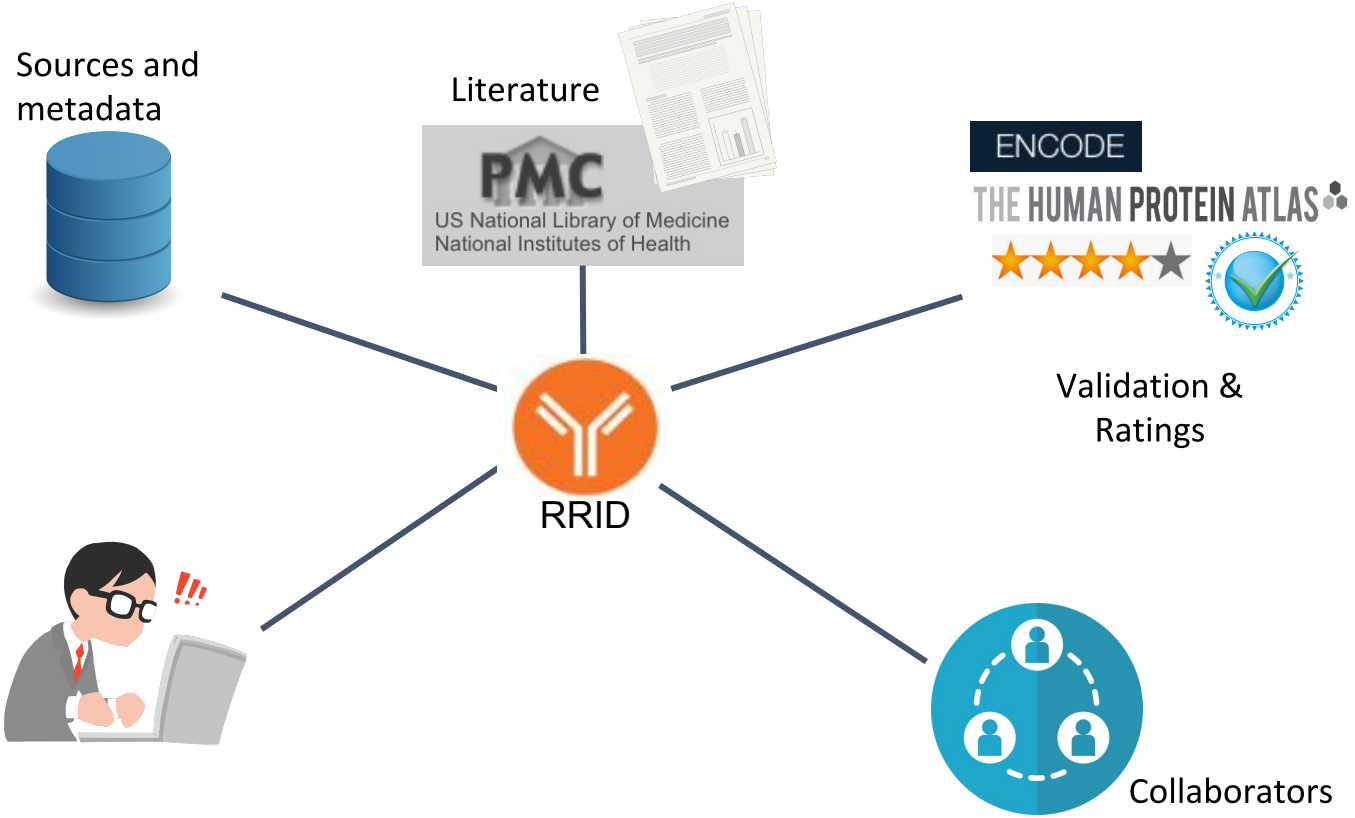
Resources Ratings, Issues and Alerts

dkNET aggregates information from multiple sources on issues, concerns and ratings (where available)



The Resource Information Network

Assembled a unique data set that aggregates information about key research resources for biomedical research using Research Resource Identifiers (**RRIDs**)



WHAT ARE RRIDS?

- A persistent identifier for research resources
- dkNET was instrumental in the development and adoption of RRIDs
- Supplied by authors to identify resources in the materials and methods section
- Designed to answer two simple questions:
  - What resources were used in a study
  - Who else has published with this resource?

RRID:AB_1855196

Anti-PYY (ab1)
antibody produced in chicken,
affinity isolated antibody

Catalog Number GW22771
Formerly listed as GenWay Catalog Number 15-288-22771,
Peptide YY Antibody.

Secondary antibod		cam
	[illegible]	Molecular Probes
Sheep IgG	No. A21448, Alexa Fluor 647	Molecular Probes
Chicken IgG	No. 703-605-155, Alexa Fluor 647	Jackson ImmunoResearch Laboratories (West Grove, PA)
Guinea pig IgG	No. 11073, Alexa Fluor 488	Molecular probes
Rabbit IgG	No. 31573, Alexa Fluor 647	Molecular probes

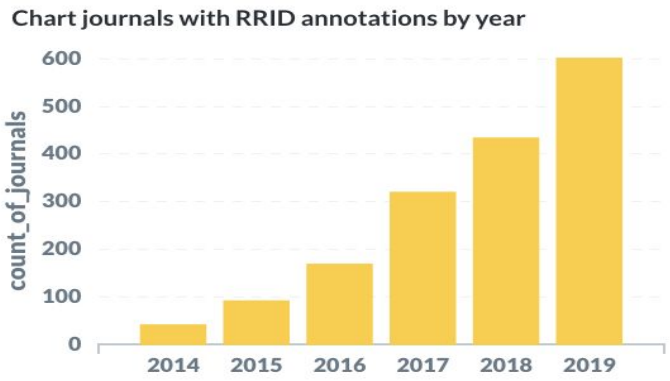
RRID Research Resource Identifier (https://dknet.org/)

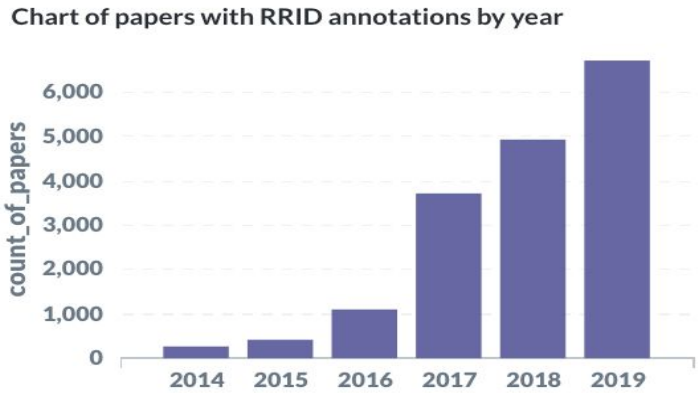
Fothergill LJ et al. Cell and Tissue Research. 375 (2) 359-69, 2019.

Growth of RRIDs

- RRIDs in use across major publishers:
  - Cell Press
  - Elsevier
  - Wiley
  - Nature
- 2019: Part of JATS standard (Journal Article Tag Suite)
- Over 212,000 resources have been identified in 22,000+ papers

+1,200
Distinct Journals

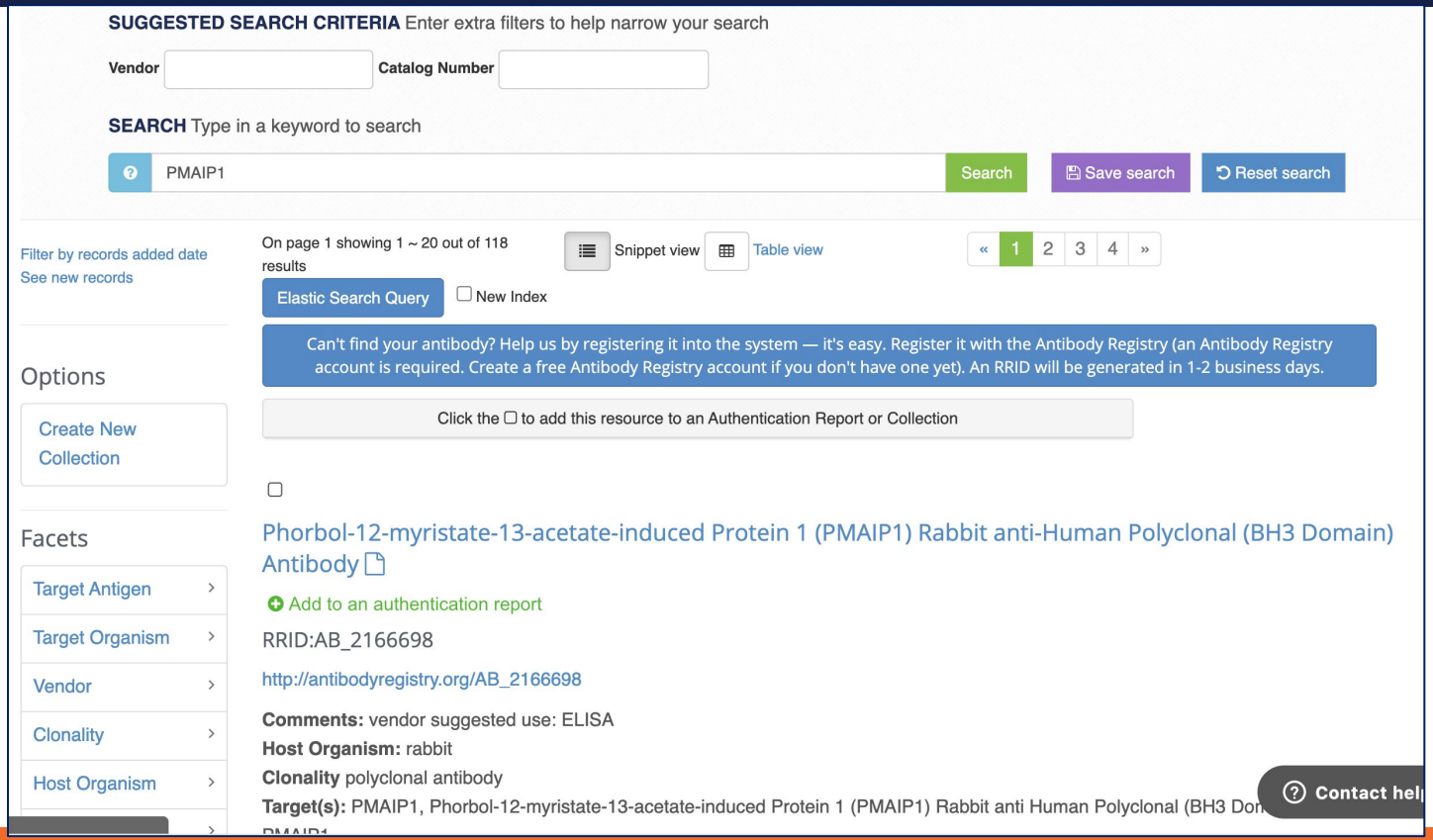




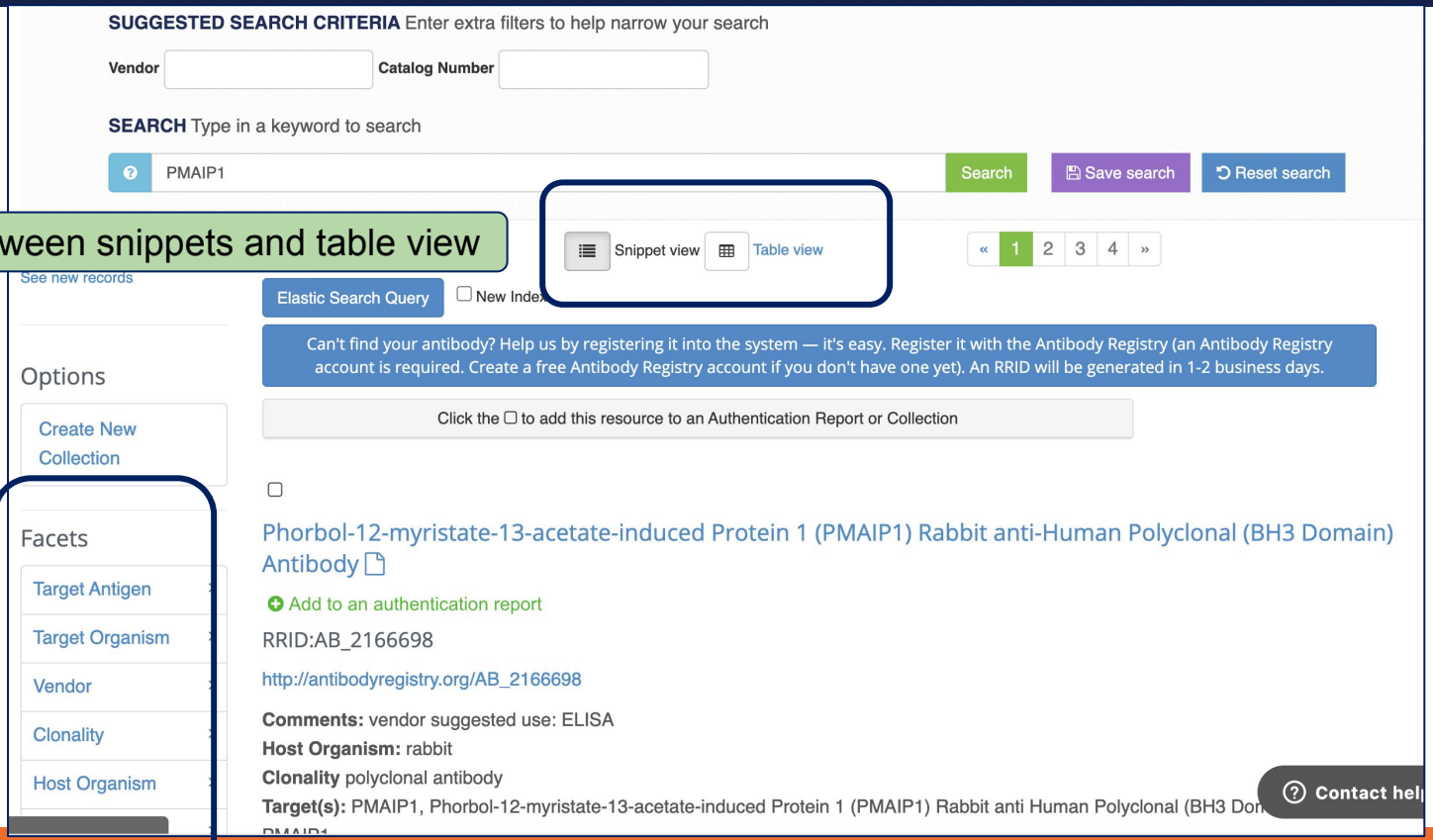
What do you really know about that antibody?

Ask dkNET!

How do I find good antibodies to PMAIP1?



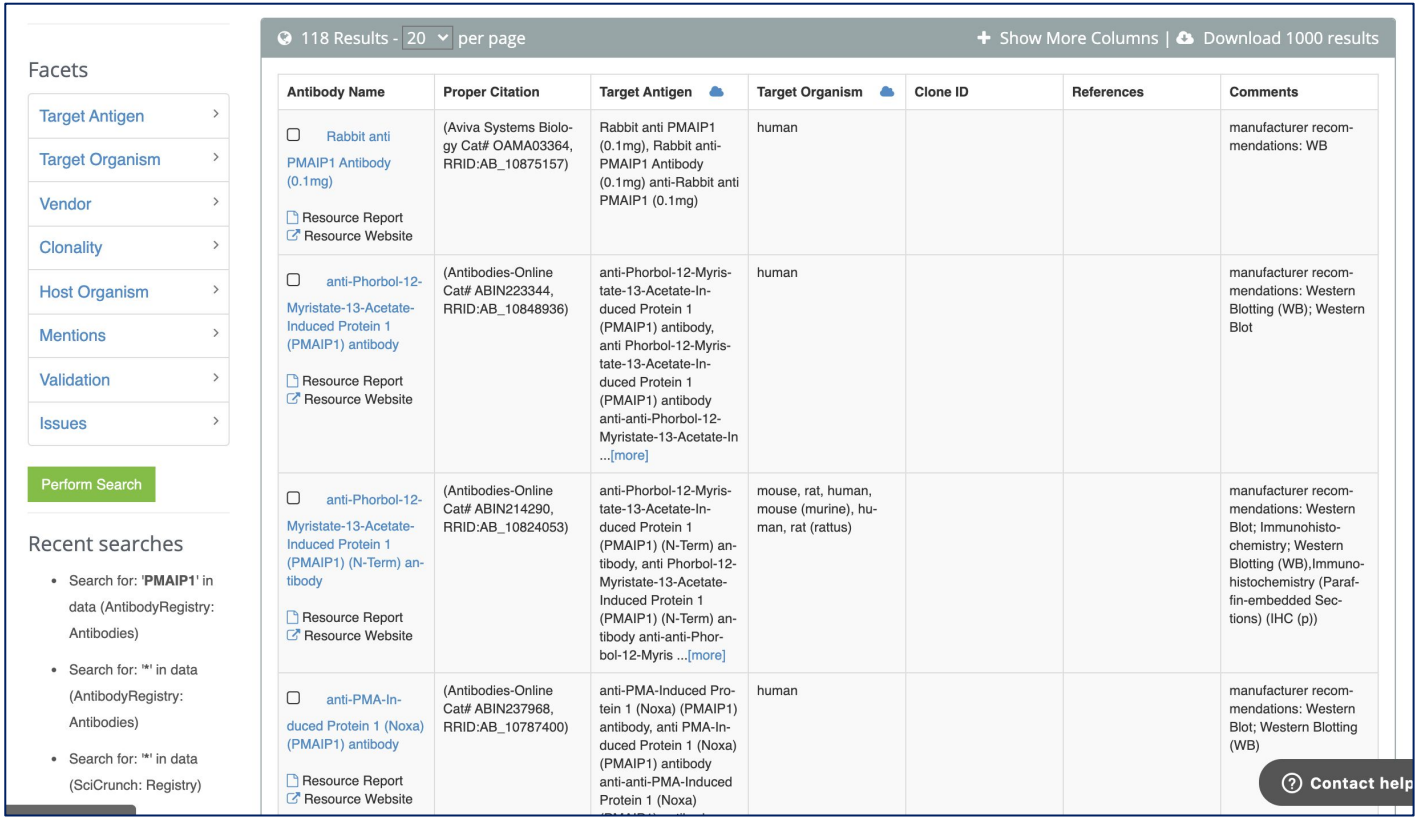
Search results



Toggle between snippets and table view

Table view and facets

- Antibody metadata
- Mentions = literature citations
- Validation information
- Issues = ratings and alerts



Tabl facets

- Antibody metadata
- Mentions = literature citations
- Validation information
- Issues = ratings and alerts

Log in for Collection Options

Facets

- Target Antigen
- Target Organism
- Vendor
- Clonality
- Host Organism
- Mentions
  - yes (2)
- Validation
  - information available (1)
- Issues
  - no known issues (104)
  - discontinued (14)

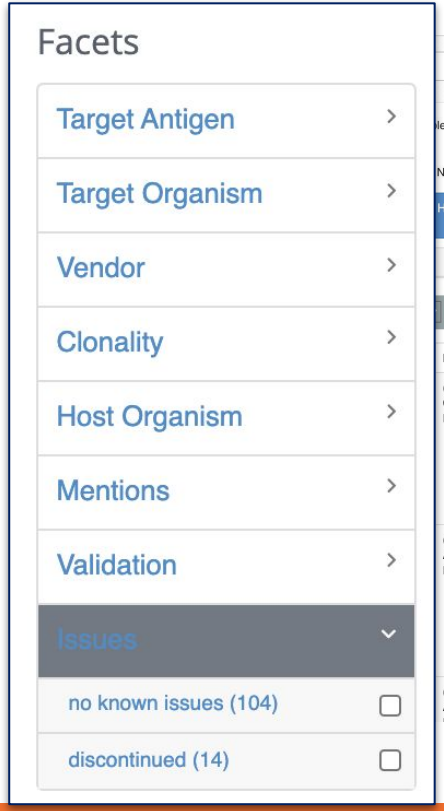
20 per page | + Show More Columns | Download 1000 results

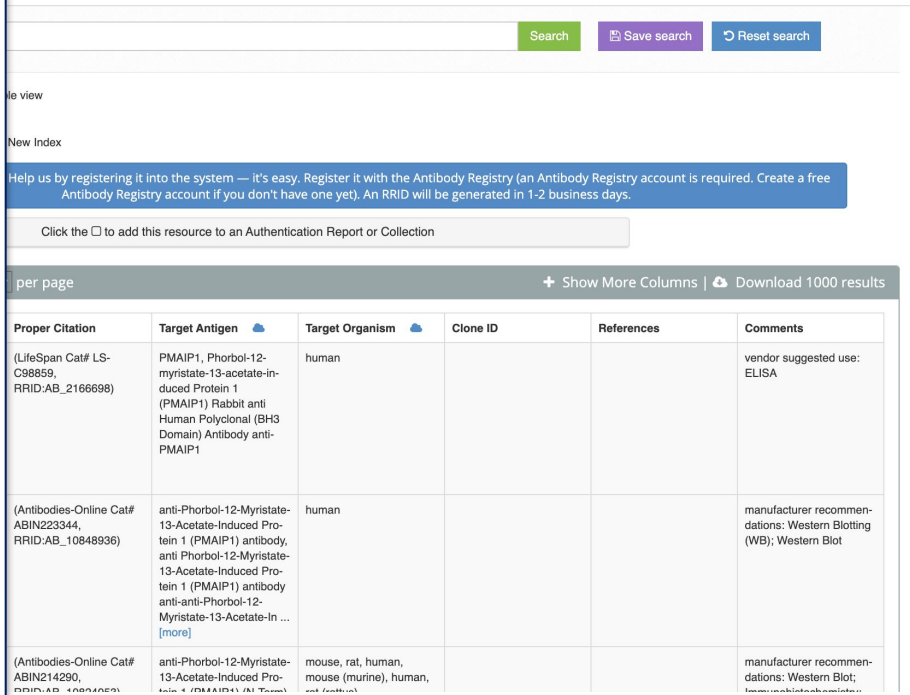
Proper Citation	Target Antigen	Target Organism	Clone ID	References	Comments
(Aviva Systems Biology Cat# OAMA03364, RRID:AB_10875157)	Rabbit anti PMAIP1 (0.1mg), Rabbit anti-PMAIP1 Antibody (0.1mg) anti-Rabbit anti PMAIP1 (0.1mg)	human			manufacturer recommendations: WB
(Antibodies-Online Cat# ABIN223344, RRID:AB_10848936)	anti-Phorbol-12-Myristate-13-Acetate-Induced Protein 1 (PMAIP1) antibody, anti Phorbol-12-Myristate-13-Acetate-Induced Protein 1 (PMAIP1) antibody anti-anti-Phorbol-12-Myristate-13-Acetate-In ...[more]	human			manufacturer recommendations: Western Blotting (WB); Western Blot
(Antibodies-Online Cat# ABIN214290, RRID:AB_10824053)	anti-Phorbol-12-Myristate-13-Acetate-Induced Protein 1 (PMAIP1) (N-Term) antibody, anti Phorbol-12-Myristate-13-Acetate-Induced Protein 1 (PMAIP1) (N-Term) antibody anti-anti-Phorbol-12-Myris ...[more]	mouse, rat, human, mouse (murine), human, rat (rattus)			manufacturer recommendations: Western Blot; Immunohistochemistry; Western Blotting (WB),Immunohistochemistry (Paraffin-embedded Sections) (IHC (p))
(Antibodies-Online Cat# ABIN237968, RRID:AB_10787400)	anti-PMA-Induced Protein 1 (Noxa) (PMAIP1) antibody, anti PMA-Induced Protein 1 (Noxa) (PMAIP1) antibody anti-anti-PMA-Induced Protein 1 (Noxa)	human			manufacturer recommendations: Western Blot; Western Blotting (WB)

Contact help

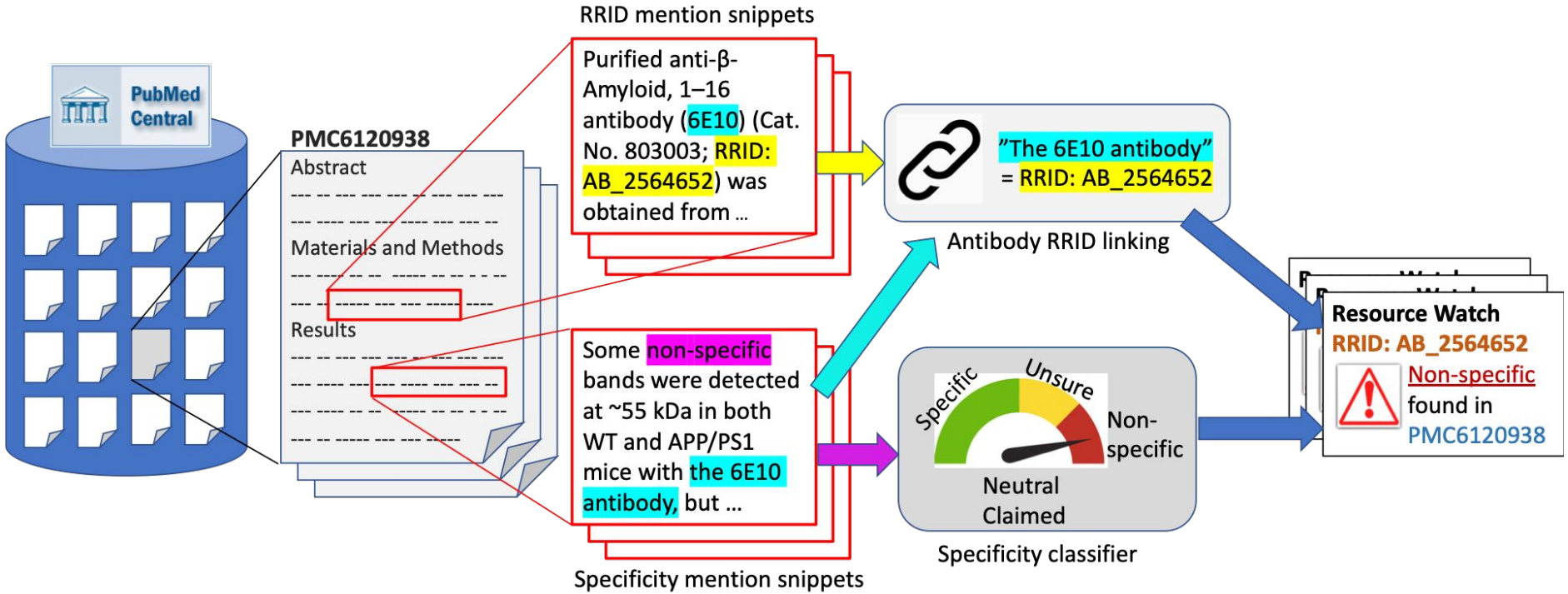
Are there any reported issues?

- dkNET aggregates alert information from multiple sources
- Building a database of issues identified from the literature = Resource Watch





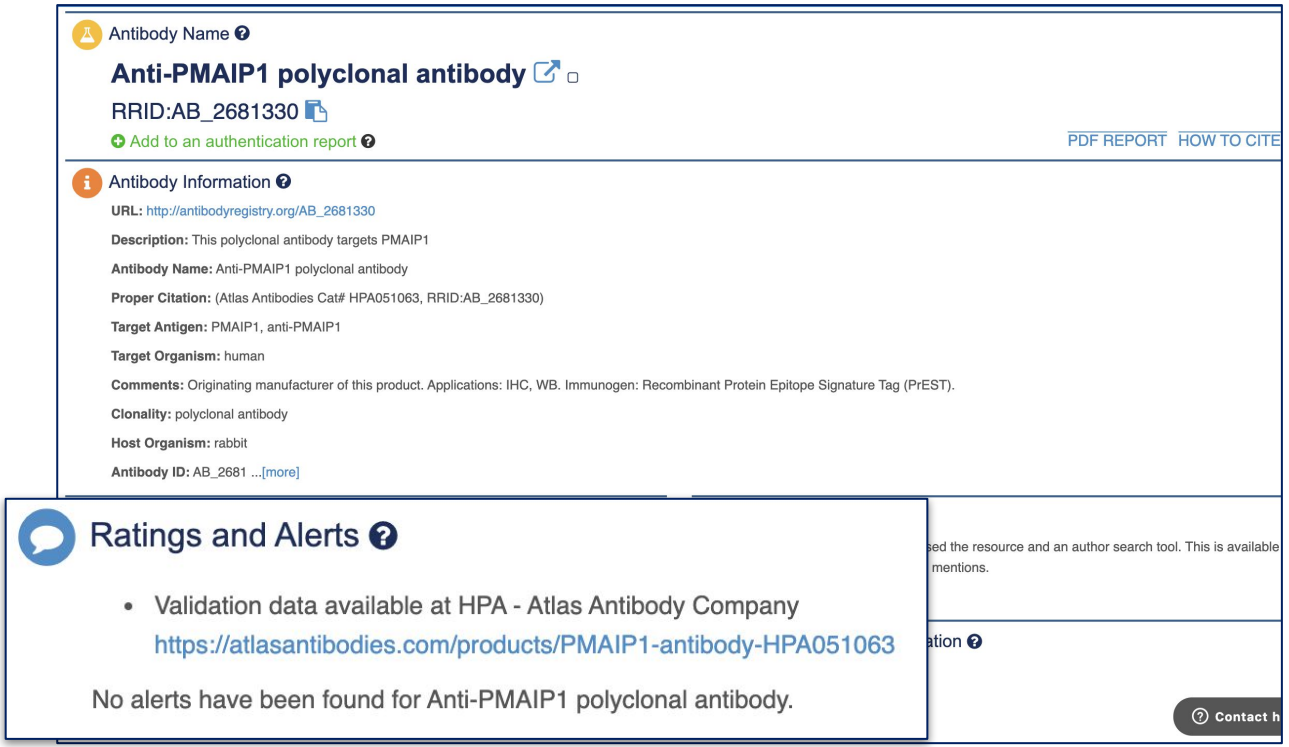
Resource Watch: Text mining issues in the literature



Drs. Chunnan Hsu and Chia-Hui Chang

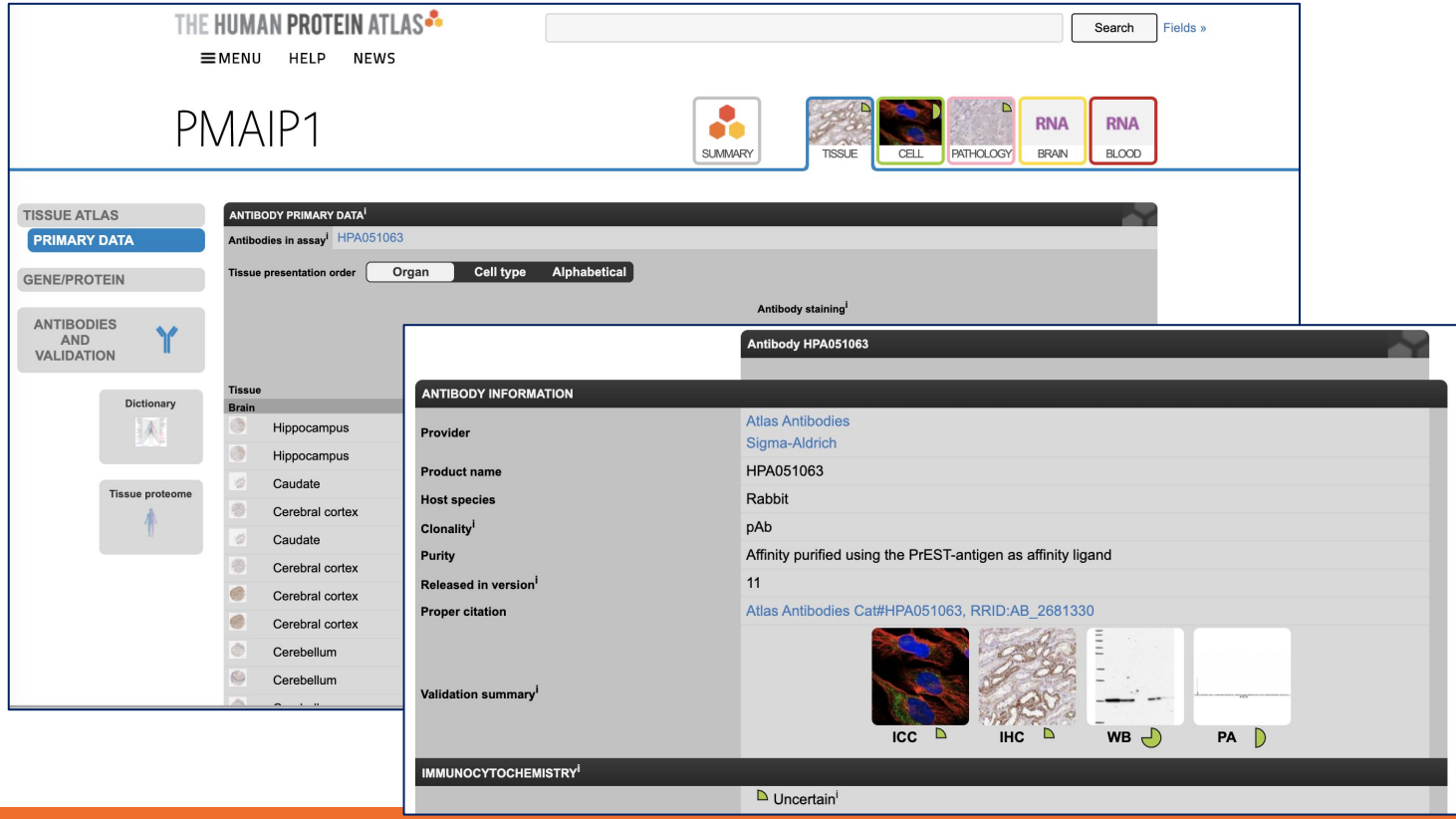
Validation information

- Antibody validation must be carried out in an application- and context-specific manner
- To validate an antibody, it must be shown to be specific, selective, and reproducible
- dkNET aggregates information from several projects that conduct careful, independent validation



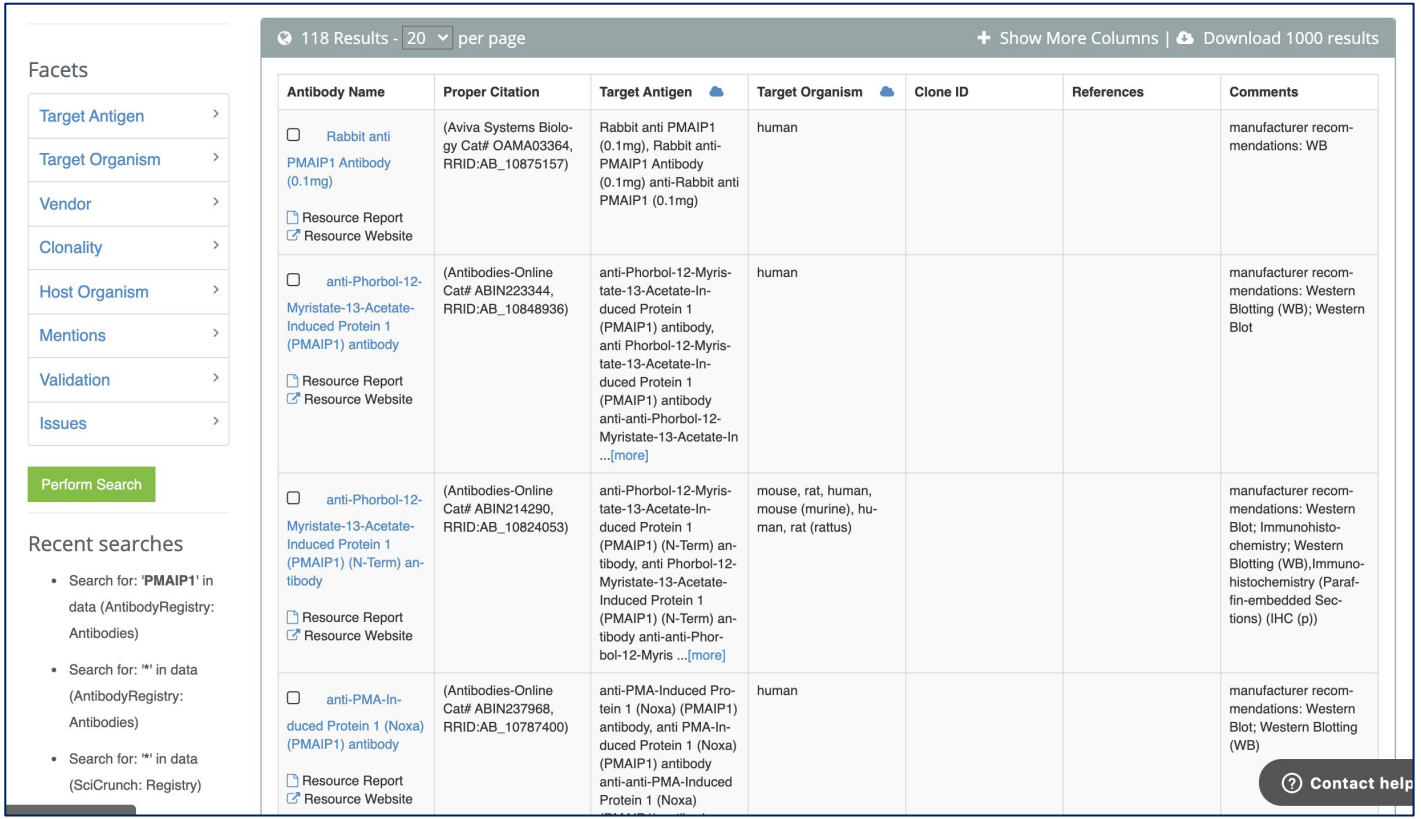
Validation information from HPA

- All antibodies used in the Human Protein Atlas are carefully validated
- Antibodies are available through Atlas antibodies



Who else has used this antibody?

- Antibody registry provides 2 types of citation:
- RRID mentions in the literature
- Supplier information



Facets

Target Antigen
Target Organism
Vendor
Clonality
Host Organism
Mentions
Validation
Issues

Perform Search

Recent searches

- Search for: '**PMAIP1**' in data (AntibodyRegistry: Antibodies)
- Search for: '*' in data (AntibodyRegistry: Antibodies)
- Search for: '*' in data (SciCrunch: Registry)

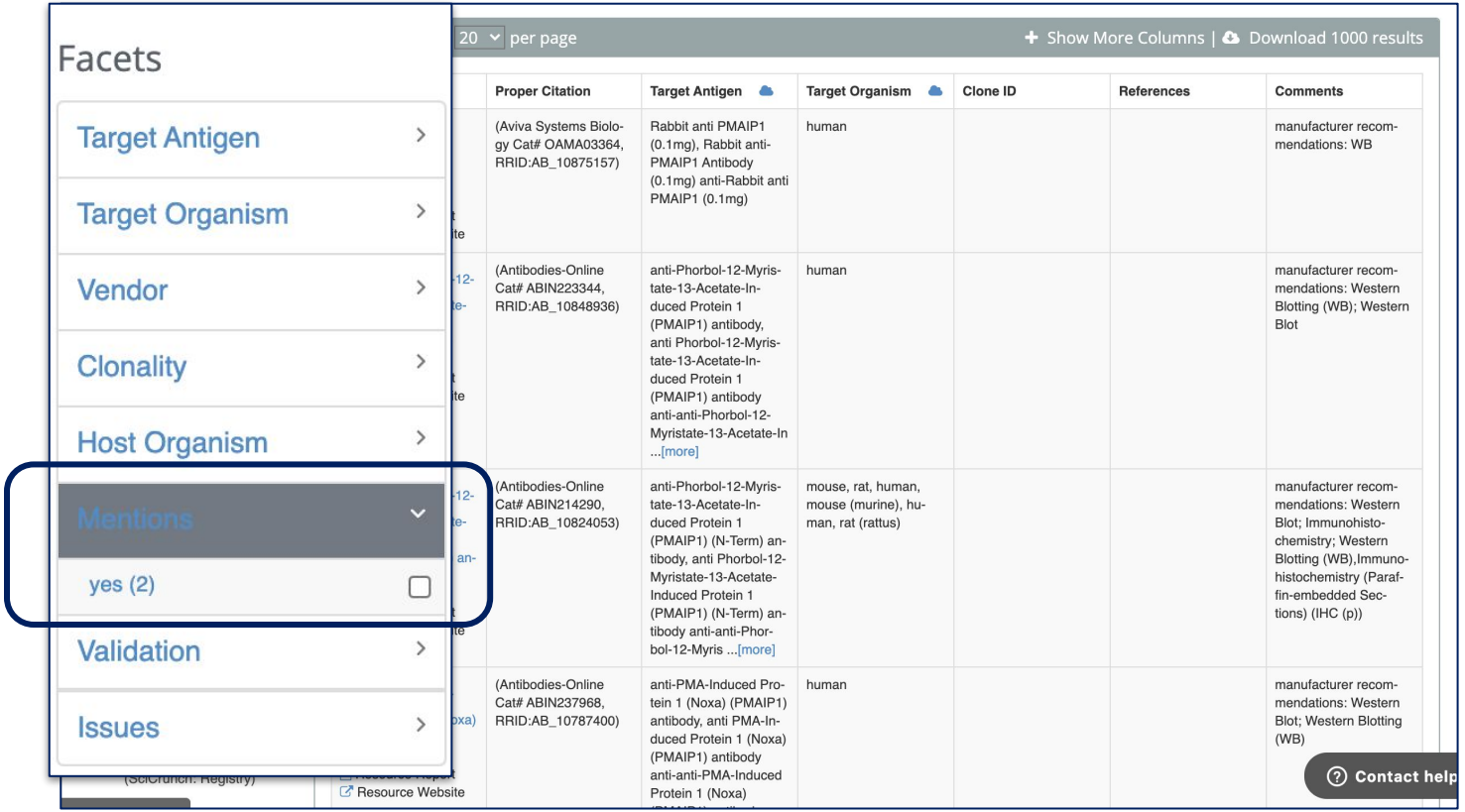
118 Results - 20 per page | + Show More Columns | Download 1000 results

Antibody Name	Proper Citation	Target Antigen	Target Organism	Clone ID	References	Comments
Rabbit anti PMAIP1 Antibody (0.1mg) Resource Report Resource Website	(Aviva Systems Biology Cat# OAMA03364, RRID:AB_10875157)	Rabbit anti PMAIP1 (0.1mg), Rabbit anti-PMAIP1 Antibody (0.1mg) anti-Rabbit anti PMAIP1 (0.1mg)	human			manufacturer recommendations: WB
anti-Phorbol-12-Myristate-13-Acetate-Induced Protein 1 (PMAIP1) antibody Resource Report Resource Website	(Antibodies-Online Cat# ABIN223344, RRID:AB_10848936)	anti-Phorbol-12-Myristate-13-Acetate-Induced Protein 1 (PMAIP1) antibody, anti Phorbol-12-Myristate-13-Acetate-Induced Protein 1 (PMAIP1) antibody anti-anti-Phorbol-12-Myristate-13-Acetate-In ...[more]	human			manufacturer recommendations: Western Blotting (WB); Western Blot
anti-Phorbol-12-Myristate-13-Acetate-Induced Protein 1 (PMAIP1) (N-Term) antibody Resource Report Resource Website	(Antibodies-Online Cat# ABIN214290, RRID:AB_10824053)	anti-Phorbol-12-Myristate-13-Acetate-Induced Protein 1 (PMAIP1) (N-Term) antibody, anti Phorbol-12-Myristate-13-Acetate-Induced Protein 1 (PMAIP1) (N-Term) antibody anti-anti-Phorbol-12-Myris ...[more]	mouse, rat, human, mouse (murine), human, rat (rattus)			manufacturer recommendations: Western Blot; Immunohistochemistry; Western Blotting (WB),Immunohistochemistry (Paraffin-embedded Sections) (IHC (p))
anti-PMA-Induced Protein 1 (Noxa) (PMAIP1) antibody Resource Report Resource Website	(Antibodies-Online Cat# ABIN237968, RRID:AB_10787400)	anti-PMA-Induced Protein 1 (Noxa) (PMAIP1) antibody, anti PMA-Induced Protein 1 (Noxa) (PMAIP1) antibody anti-anti-PMA-Induced Protein 1 (Noxa)	human			manufacturer recommendations: Western Blot; Western Blotting (WB)

Contact help

Who else has used this antibody?

- Antibody registry provides 2 types of citation:
- RRID mentions in the literature
- Supplier information



Who else has used this antibody?

2 Results - 20 per page | + Show More Columns | Download 1000 results

Antibody Name	Proper Citation	Target Antigen	Target Organism	Clone ID	References	Comments
NOXA (114C307) antibody Resource Report Resource Website 1+ mentions	(Santa Cruz Biotechnology Cat# sc-56169, RRID:AB_784877)	Human PMAIP1, anti-Human PMAIP1	human	114C307	PMID:25392500 PMID:29894692 PMID:31543463	validation status unknown check with seller; recommendations: Immunoprecipitation; Western Blot; Western Blotting, Immunoprecipitation
Anti-Noxa Mouse mAb (114C307) antibody Resource Report Resource Website 1+ mentions	(Millipore Cat# OP180, RRID:AB_2268468)	PMAIP1, anti-PMAIP1	human		PMID:29932898 PMID:31455158 PMID:31940492	seller recommendations: western blot

Mentions and Co-mentions

Antibody Name

NOXA (114C307) antibody

RRID:AB_784877

Add to an authentication report

Antibody Information

URL: http://antibodyregistry.org/AB_784877

Description: This monoclonal antibody targets Human PMAIP1

Antibody Name: NOXA (114C307) antibody

Proper Citation: (Santa Cruz Biotechnology Cat# sc-56169, RRID:AB_784877)

Target Antigen: Human PMAIP1, anti-Human PMAIP1

Target Organism: human

Clone ID: 114C307

References: PMID:25392500, PMID:29894692, PMID:31543463

Comments: validation status unknown check with seller; recommendations: Immunopr

Usage and Citation Metrics

We found 3 mentions in open access literature.

View full usage report

Most recent articles:

Guièze R, et al. (2019) Mitochondrial Reprogramming Underlies Resistance to BCL-2 Inhibition in Lymphoid Malignancies. Cancer cell, 36(4), 369-384.e13. (PMID:31543463)

Dhar D, et al. (2018) Liver Cancer Initiation Requires p53 Inhibition by CD44-Enhanced Growth Factor Signaling. Cancer cell, 33(6), 1061-1077.e6. (PMID:29894692)

Chen Y, et al. (2014) Regulation of neuronal gene expression and survival by basal NMDA receptor activity: a role for histone deacetylase 4. The Journal of neuroscience : the official journal of the Society for Neuroscience, 34(46), 15327-39. (PMID:25392500)

*NOTICE: Multiple vendors found, please select your vendor: Santa Cruz Biotechn

NOXA (114C307) antibody

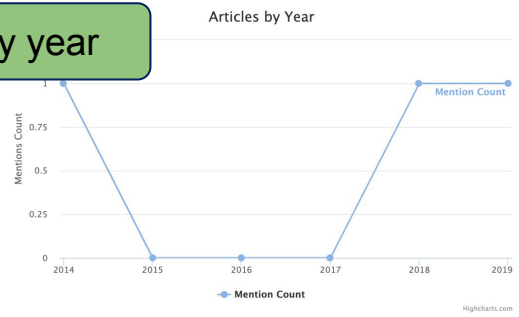
RRID:AB_784877

Add to an authentication report

DOWNLOAD MENTIONS

Usage

Mentions by year



* Resource mentions from this and last year may still be in process.

About this Report

The report results are from **Research Resource Identifier (RRID)** - based text mining of the literature of PubMed Central (PMC) Open Access Subset (OA Subset).

For tools, we also include additional results from searching for resource name and URL in the PMC OA Subset. See Ozyurt, et. al., PMID:26730820

Check Google Scholar for al

Co-Mentions

Other research resources frequently mentioned with this resource

*Please note that when co-mention number is small, resources listed here do not mean that they are frequently used together. We are also aware that commercial organizations are in the list and we are currently working on improving this service by removing these organizations.

- Bax Antibody
- AMPK alpha (D5A2) Rabbit mAb antibody
- Anti-Bim Antibody, Unconjugated
- BID Antibody (Mouse Specific)
- PKA beta (catalytic subunit) antibody

Additional Report Filters

Filter by Publication Year Input year

Find mentions based on location BETA TESTING

City State/Region/Province

Search

Search using your location

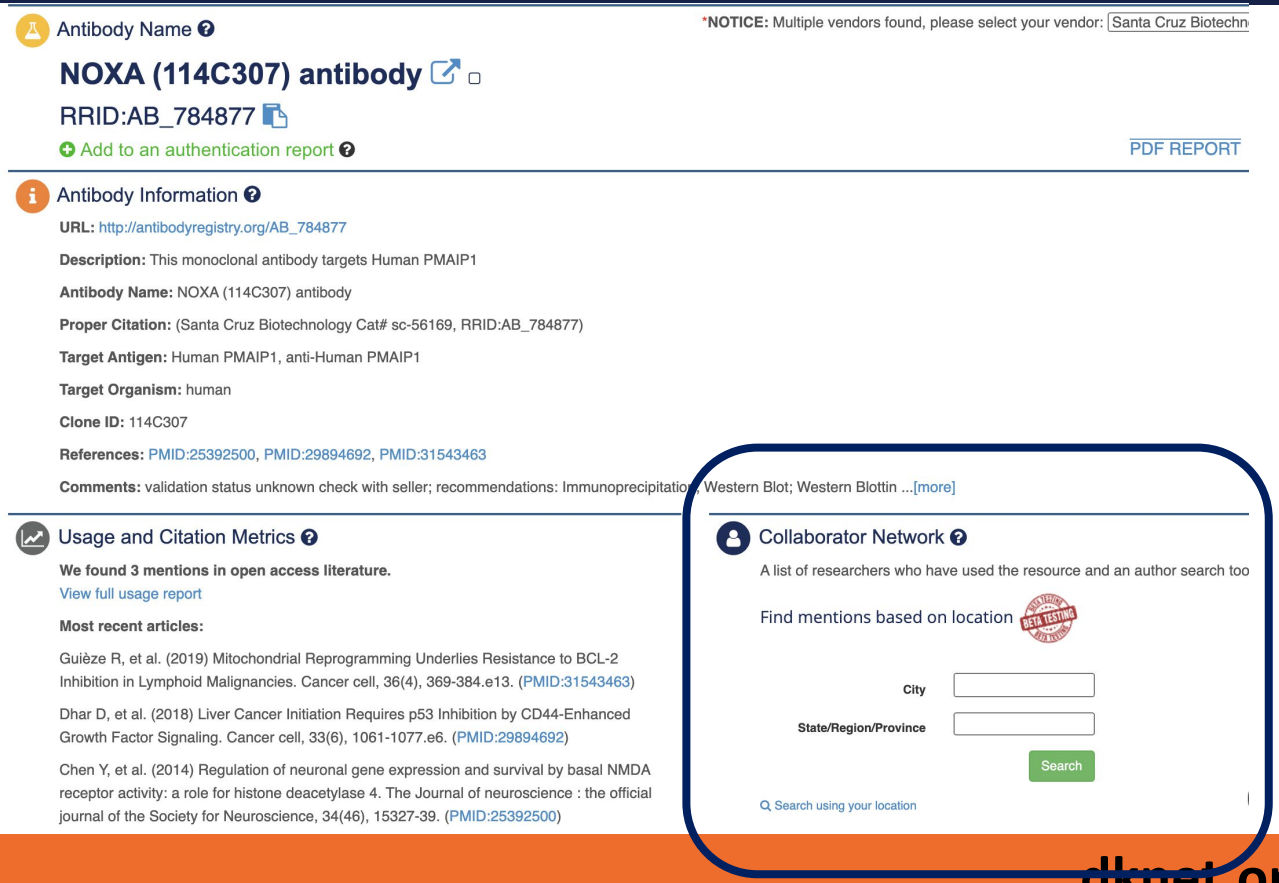
Contact help

Search

Search using your location

Collaborator network (very beta)

- Find colleagues near you who might have experience with this antibody



Can I borrow some antibody?

Collaborator Network

A list of researchers who have used the resource and an author search tool

Find mentions based on location BETA TESTING

City

State/Region/Province

Search using your location

Enter a city or state or search using your location

All Mentions (1 mentions) [Download Mentions]

Near you

First Previous 1 Next Last Page 1 of 1 (1 ~ 1 of 1)

- Dhar D, et al. (2018) Liver Cancer Initiation Requires p53 Inhibition by CD44-Enhanced Growth Factor Signaling. Cancer cell,
  - Debanjan Dhar - San Diego, California, United States of America
  - Laura Antonucci - San Diego, California, United States of America
  - Ju Youn Kim - San Diego, California, United States of America
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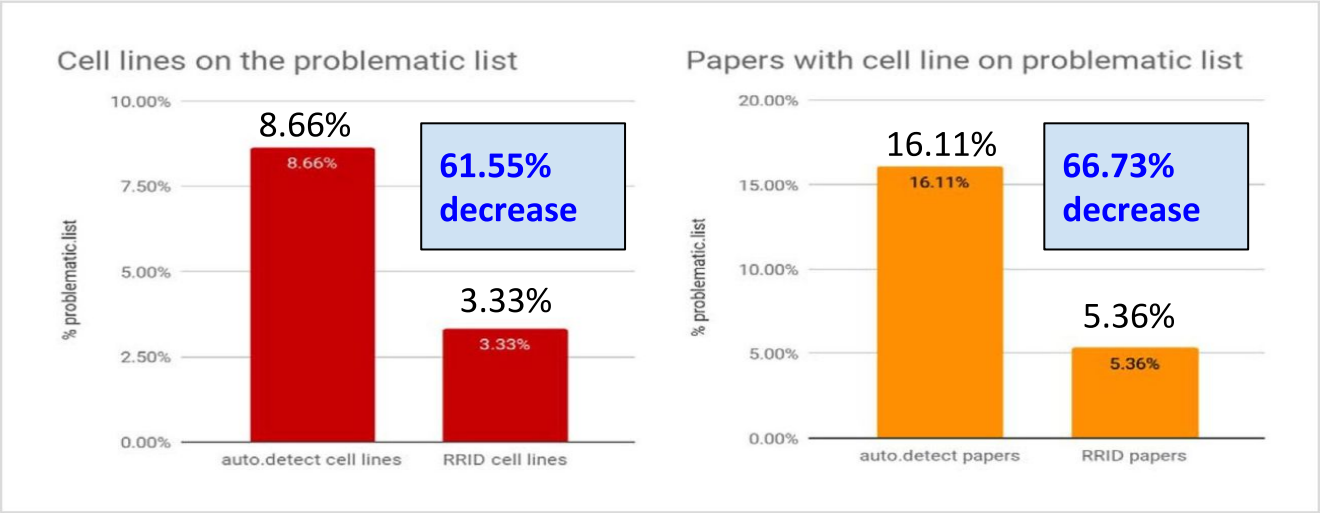
Know before you go...

Measuring the impact of RRIDs and the RIN

Use of RRIDs associated with a lower reported use of problematic cell lines



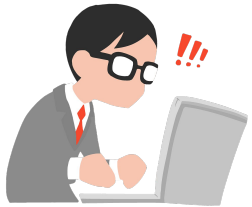
Percentages of papers with cell lines found on the problematic list.

The "auto.detect.cell" lines data come from the edit distance metric, same as Figure 2; n=305,161; the RRID cell lines are based on 1,502 cell lines. The "auto.detect" papers percentage is based on n=150,459 unique papers, where the problematic cell-line list is detected based on the edit distance metric. The RRID papers percentage is based on n=634 papers.

Babic Z, et al. eLIFE 2019; 8:e41676 DOI: 10.7554/eLife.41676

The Resource Information Network is inserted into the Publication Process

- RRIDs stand between authors and publications
- Authors encounter information when they obtain RRID through dkNET or the Resource Identification Portal, which are issued by over 25 distinct authorities for different types of resources
- Powered by dkNET



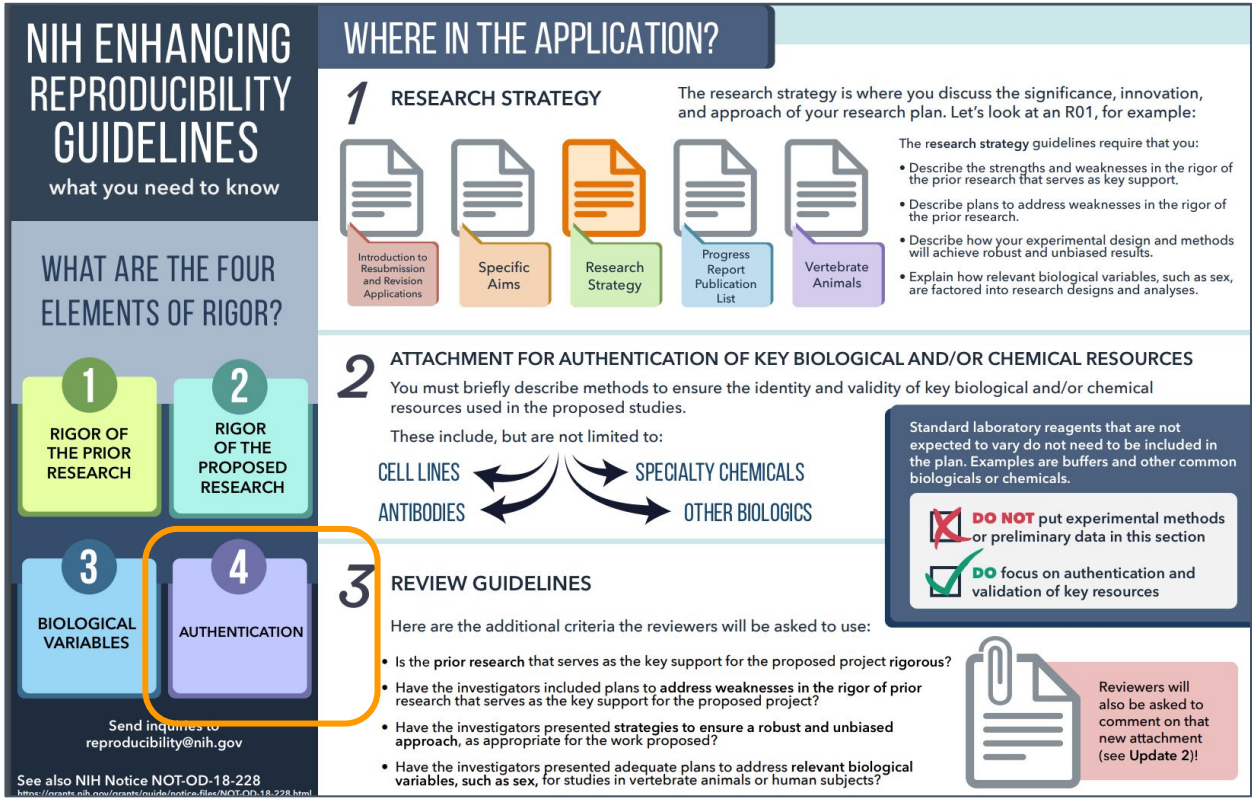
Authentication Reports

1. Alert researchers of any issues with cell lines and antibodies ***before*** the study is performed
2. Compliance with the NIH requirements for grant applications

- dkNET has created a tool to help comply with funder mandates and best practices for authenticating key biological resources

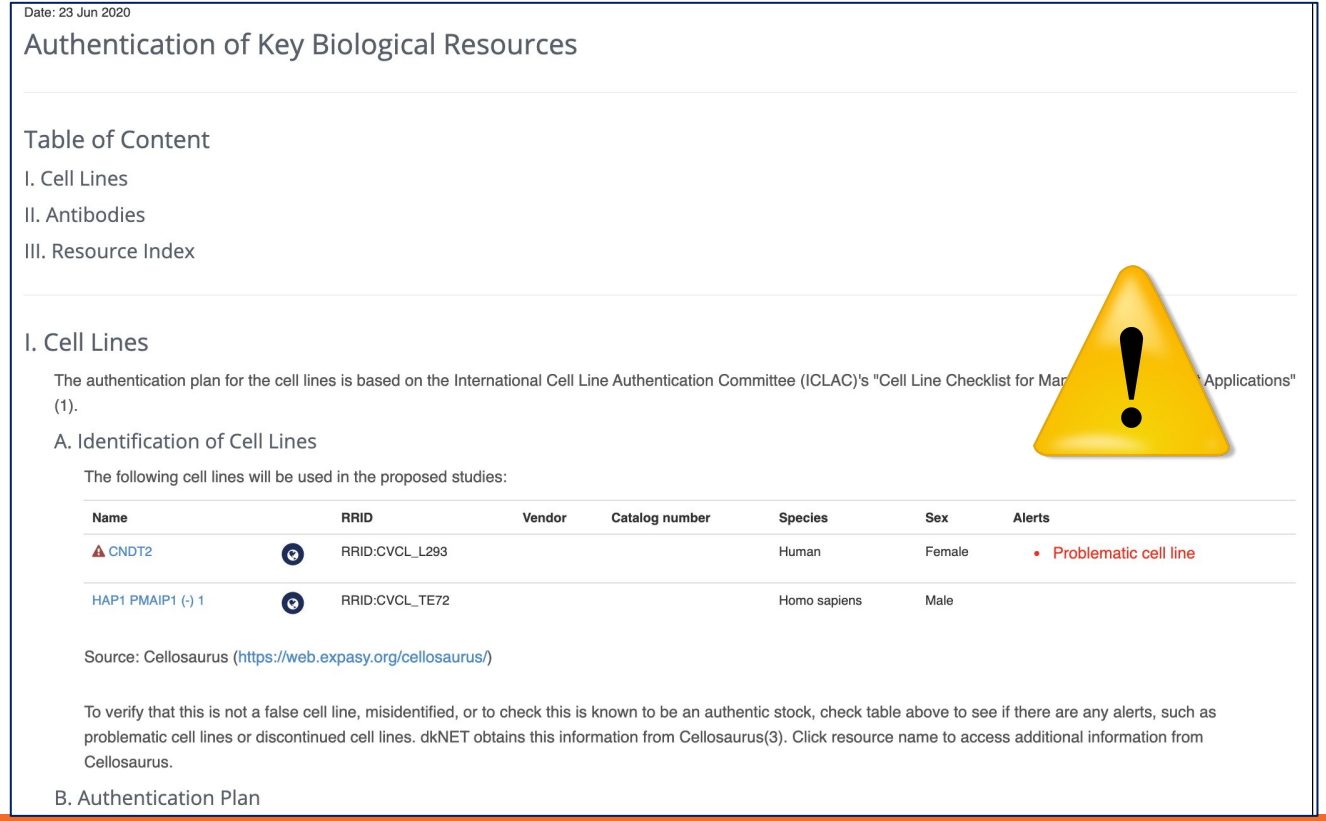
NIH Rigor & Reproducibility Guidelines

When preparing a grant proposal for the US National Institutes of Health, researchers must provide a plan for authenticating key research resources



Custom Authentication Reports

- Select cell lines and antibodies you are planning to use
- dkNET creates a custom report
- Displays alerts and recommendations for authentication of cell lines and antibodies



Date: 23 Jun 2020

Authentication of Key Biological Resources

Table of Content

I. Cell Lines

II. Antibodies

III. Resource Index

I. Cell Lines

The authentication plan for the cell lines is based on the International Cell Line Authentication Committee (ICLAC)'s "Cell Line Checklist for Mar Applications" (1).

A. Identification of Cell Lines

The following cell lines will be used in the proposed studies:

Name	RRID	Vendor	Catalog number	Species	Sex	Alerts
CNDT2	RRID:CVCL_L293			Human	Female	• Problematic cell line
HAP1 PMAIP1 (-) 1	RRID:CVCL_TE72			Homo sapiens	Male	

Source: Cellosaurus (https://web.expasy.org/cellosaurus/)

To verify that this is not a false cell line, misidentified, or to check this is known to be an authentic stock, check table above to see if there are any alerts, such as problematic cell lines or discontinued cell lines. dkNET obtains this information from Cellosaurus(3). Click resource name to access additional information from Cellosaurus.

B. Authentication Plan

Antibody report

- Walks through common application errors

II. Antibodies

This authentication plan for antibodies is based on the methods suggested in "A proposal for validation of antibodies" (Uhlen M et. al., 2016)(1), the guideline published in the Journal of Comparative Neurology (Saper C, 2005)(2), and the Example Authentication of of Key Biological and/or Chemical Resources (Bandrowski A)(3).

A. Identification of Antibodies

The following antibodies will be used in the proposed studies:

Name	RRID	Vendor	Catalog Number	Target Organism	Comments	Alerts
Anti-SSRP1 polyclonal antibody	RRID:AB_1857509	Atlas Antibodies	HPA002697	human	Originating manufacturer of this product. Applications: IHC, WB. Immunogen: Recombinant Protein Epitope Signature Tag (PrEST).	
NOXA (114C307) antibody	RRID:AB_784877	Santa Cruz Biotechnology	sc-56169	human	validation status unknown check with seller; recommendations: Immunoprecipitation; Western Blot; Western Blotting, Immunoprecipitation	

Source: Antibody Registry (http://antibodyregistry.org/)

Check table above to see if there are any warning signs, such as discontinued antibodies, to determine whether there are any known issue. dkNET obtains this information from the Antibody Registry(5). Click resource name to access additional information from the Antibody Registry.

B. Validation

a. Antibody validation must be carried out in an application- and context-specific manner (Uhlen et al., 2016), i.e., just because you used an antibody successfully in one application, doesn't mean it will translate. To validate an antibody, it must be shown to be specific, selective, and reproducible in the context for which it is to be used (Bordeaux et al., 2014). See b. Suggested validation methods. When selecting an antibody to use, pay special attention to the following:

i. **Check Target Organism** - Check to see if the antibody has been developed for and tested in the target species for your experiment in the Target Organism field.
ii. **Check Application** - Check if your planned applications included in the recommended applications provided by the vendor listed in the Comments field. We do not recommend using an antibody that has not been tested for a specific application.
iii. **Check Validation Information** - Check validation information to see if it is a widely used reagent or if any concerns have been raised.

Help with validation methods

b. Suggested validation methods based on applications(1)

Validation strategy	Genetic	Orthogonal	Independent antibody	Tagged protein expression	IMS
Validation principle	The expression of the target protein is eliminated or significantly reduced by genome editing or RNA interference	Expression of the target protein is compared with an antibody-independent method	Expression of the target protein is compared using two antibodies with nonoverlapping epitopes	The target protein is expressed using a tag, preferably expressed at endogenous levels	The target protein is captured using an antibody and analyzed using MS
Validation criteria	Elimination or significant reduction in antibody labeling after gene disruption or mRNA knockdown Search dkNET for knockout organism	Significant correlation of protein levels detected by an antibody and an orthogonal method (e.g., MS)	Significant correlation of protein levels detected by two different antibodies recognizing independent regions of the same target protein	Significant correlation between antibody labeling and detection of the epitope tag	Target protein peptides among the most abundant detected by MS following immunocapture
Suitable for these applications	WB, IHC, ICC, FS, SA, IP/ChIP, RP	WB, IHC, ICC, FS, SA, RP	WB, IHC, ICC, FS, SA, IP/ChIP, RP	WB, IHC, ICC, FS	IP/ChIP

WB, western blot; IHC, immunohistochemistry; ICC, immunocytochemistry, including immunofluorescence microscopy; FS, flow sorting and analysis of cells; SA, sandwich assays, including ELISA; IP, immunoprecipitation; ChIP, chromatin immunoprecipitation; and RP, reverse-phase protein arrays.

Summary

- dkNET has assembled a unique index, the Resource Information Network, that aggregates information about research resources and how they perform
- Resource Reports and Authentication Plans utilize the RIN to make this information available to researchers to help choose the right reagents and support reproducibility
- Coming soon:
  - Resource Watch-expanded database of reported issues with research resources
  - Expanded services for Authentication Plans

The dkNET team

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