Authentication Report

Go back to report | Download PDF **Please note that for NIH grant applications, the plan should be no more than one page**. You can copy and paste the information and edit the information in a word file.

Date: 29 Apr 2019

Authentication of Key Biological Resources

Table of Content

I. Cell Lines

Authentication plan for the cell lines is based on International Cell Line Authentication Committee (ICLAC)'s "Cell Line Checklist for Manuscripts and Grant Applications"(1). Follow these steps to prepare the attachment of Authentication of key biological and/or chemical resources(2) in the grant application.

A. Identification of Cell Lines

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

Name	RRID	Vendor	Catalog number	Species	Sex	Alerts
Hep-G2	RRID:CVCL_0027	TKG	TKG 0205	Homo sapiens	Male	• Problematic cell line • Discontinued cell line
hAG	RRID:CVCL_8223			Homo sapiens	Female	• Problematic cell line

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 aggregates the information from Cellosaurus(3). Click resource name to get access to more information.

B. Authentication Plan

The gold standard for authentication testing of cell lines is Short Tandem Repeat (STR) profiling. STR profiling should be performed and compared to results from donor tissue, or to online databases of cell line STR profiles. Authentication testing should be performed on established cell lines regardless of the application, and the test method and results included in the Materials and Methods section. Testing should be done, at minimum, at the beginning and end of experimental work(4, 5)

Sources:

1. Cell Line Checklist for Manuscripts and Grant Applications, International Cell Line Authentication Committee (ICLAC), version 1.2, updated on May 9, 2014.
2. Guidance: Rigor and Reproducibility in Grant Applications
3. Cellosaurus (https://web.expasy.org/cellosaurus/) is a cell line knowledge resource and nomenclature authority. Cellosaurus covers the information of "ICLAC register of misidentified cell lines" (latest version 8.0, released Dec. 1, 2016,) and provides more updated information.
4. Standards for Cell Line Authentication and Beyond, The National Institute of Standards and Technology (NIST).
5. Published standard: ANSI/ATCC ASN-0002-2011, Authentication of Human Cell Lines: Standardization of STR Profiling, American National Standards Institute (ANSI).

II. Antibodies

This authentication plan of 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). Follow these steps to prepare the attachment of Authentication of key biological and/or chemical resources(4) in the grant application.

A. Identification of Antibodies

The following antibodies will be used in the proposed studies:

Name	RRID	Vendor	Catalog Number	Target Organism	Comments	Alerts
TGF beta-1 Antibody	RRID:AB_2255874	Thermo Fisher Scientific	PA1-24735	human	Discontinued; Applications: ELISA (0.5-1.0 ug/ml), Inhibition Assays, Western Blot (0.1-0.2 ug/ml); Reactive Species: Human	• Discontinued antibody

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

Check table above to see if there are any warning signs, such as discontinued antibodies, to determine if other investigators who used this antibody raised issues. dkNET aggregates the antibody information from Antibody Registry(5). Click resource name to get access to more information.

B. Validation

a. Check **Target Organism**, **Application**, and **Validation Information**

i. **Check Target Organism** - Check if the target organisms in your planned experiments are listed in the Target Organism field. If they are different, you need to authenticate the specificity (see b. Suggested validation methods) in addition to appropriate experiment controls.
ii. **Check Application in Comment field** - Check if your planned applications are different from the applications listed in the Comments field. If they are different, you need to authenticate the specificity (see b. Suggested validation methods) in addition to appropriate experiment controls.
iii. **Check Validation Information in Comment field** - Check if the validation information is available in the Comments field. If the validation information is not available or unknown, you need to authenticate the specificity (see b. Suggested validation methods) in addition to appropriate experiment controls.

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.

Sources:

1. Uhlen M et. al. A proposal for validation of antibodies. Nature Methods, Oct;13(10):823-7. 2016.
2. Saper C. An open letter to our readers on the use of antibodies. Journal of comparative neurology, 493(4):477-8, 2005.
3. Bandrowski A. Example Authentication of of Key Biological and/or Chemical Resources, NIH Policy on Rigor and Reproducibility Section, UC San Diego Library website.
4. AntibodyRegistry (https://antibodyregistry.org)
5. Guidance: Rigor and Reproducibility in Grant Applications, National Institute of Health Office of Extramural Research Website.

III. Resource Index

Hep-G2

Type: Cell line

Data:

Name:	Hep-G2
Proper Citation:	(TKG Cat# TKG 0205, RRID:CVCL_0027)
ID:	CVCL_0027
Organism:	Homo sapiens
Disease:	Hepatoblastoma
Comments:	Problematic cell line: Misidentified. Originally thought to be a hepatocellular carcinoma cell line but shown to be from an hepatoblastoma (PubMed=19751877). Part of: Cancer Cell Line Encyclo ...[more]
References:	DOI:10.1101/378497, DOI:10.11418/jtca1981.16.3_173, DOI:10.1385/CP:1:3-4:313, Patent:US4393133, PMID:233137, PMID:2439335, PMID:8050184, PMID:8384076, PMID:8389256, PMID:11050057, PMID:120296 ... [more]
Category:	Cancer cell line
Sex:	Male
Synonyms:	HEP-G2, Hep G2, HEP G2, HepG2, HEPG2
Vendor:	TKG
Catalog Number:	TKG 0205
Cross References:	BTO:0000599, CLO:0003704, CLO:0003713, CLO:0050856, CLO:0050858, EFO:0001187, MCCL:MCC:0000222, CLDB:cl1635, CLDB:cl1636, CLDB:cl1637, CLDB:cl1638, CLDB:cl1639, CLDB:cl1641, CLDB:cl1642, CLDB ...[more]
Hierarchy:	
Originate from Same Individual:	

- Problematic cell line
- Discontinued cell line

hAG

Type: Cell line

Data:

Name:	hAG
Proper Citation:	(RRID:CVCL_8223)
ID:	CVCL_8223
Organism:	Homo sapiens
Disease:	Bladder carcinoma
Comments:	Problematic cell line: Contaminated. Shown to be a T24 derivative (PubMed=10508494; PubMed=20143388). Registration: International Cell Line Authentication Committee, Register of Misidentifie ...[more]
References:	PMID:7787432, PMID:10508494, PMID:20143388
Category:	Cancer cell line
Sex:	Female
Synonyms:	HAG, human Adenomatous Goiter
Vendor:	
Catalog Number:	
Cross References:	BioSample:SAMN03151941, Wikidata:Q54872367
Hierarchy:	CVCL:0554
Originate from Same Individual:	

- Problematic cell line

TGF beta-1 Antibody

Type: Antibody

Data:

Antibody Name:	TGF beta-1 Antibody
Proper Citation:	(Thermo Fisher Scientific Cat# PA1-24735, RRID:AB_2255874)
Target Antigen:	TGF beta-1, anti-TGF beta-1
Target Organism:	human
Clone ID:	
References:	
Comments:	Discontinued; Applications: ELISA (0.5-1.0 ug/ml), Inhibition Assays, Western Blot (0.1-0.2 ug/ml); Reactive Species: Human
Clonality:	polyclonal antibody
Host Organism:	chicken
Antibody ID:	AB_2255874
Vendor:	Thermo Fisher Scientific
Catalog Number:	PA1-24735

- Discontinued antibody