Investigation File Example

[Ontology Source Reference] [Investigation] [Investigation Publications] [Investigation Contacts] [Material] [Study] [Study Design Descriptors] [Study Publications] [Study Factors] [Study Assays] [Study Protocols] [Study Contacts]

This page provides an example of the ISA-TAB-Nano Investigation File leveraging data from an Nanotechnology Characterization Laboratory (NCL) Investigation (*NCL200612A*). For the complete file, refer the NCL Investigation File Example.

The ISA-TAB-Nano Investigation File consists of the following sections:

- ONTOLOGY SOURCE REFERENCE
- INVESTIGATION
- INVESTIGATION PUBLICATIONS
- INVESTIGATION CONTACTS
- MATERIAL
- STUDY
    - STUDY DESIGN DESCRIPTORS
    - STUDY PUBLICATIONS
    - STUDY FACTORS
    - STUDY ASSAYS
    - STUDY PROTOCOLS
    - STUDY CONTACTS

The following sections provide an example of each Investigation File section.

Ontology Source Reference

Example Investigation File Ontology Source Reference Section

A	B	C	D	E	F	G
Term Source Name	MO	NPO	UO	ChEBI	PATO	NCIt
Term Source File	http://purl.bioontology.org/ontology/MO	http://purl.bioontology.org/ontology/npo	http://purl.bioontology.org/ontology/UO	http://purl.bioontology.org/ontology/CHEBI	http://purl.bioontology.org/ontology/PATO	http://ncit.nci.nih.gov/
Term Source Version	v.1.3.1.1	v.2011-02-12		v.80		v.11.11d
Term Source Description	MGED Ontology	NanoParticle Ontology	Unit Ontology	Chemical Entities of Biological Interest	Phenotype Ontology	NCI Thesaurus

Investigation

Example Investigation File Investigation Section

A	B
Investigation Identifier	NCL200612A
Investigation Title	Dendrimer-Based MRI Contrast Agents
Investigation Description	The goal of this investigation is to characterize a PAMAM dendrimer with an associated gadolinium chelate MRI contrast agent.
Investigation Submission Date	2002-11-30
Investigation Public Release Date	2002-11-30
Investigation Disease	
Investigation Disease Term Accession Number	
Investigation Disease Term Source REF	
Investigation Outcome	

Investigation Publications

Example Investigation Publication Section

A	B
Investigation PubMed ID	18095846
Investigation Publication DOI	10.2217/17435889.2.6.789
Investigation Publication Author List	Hall JB; Dobrovolskaia MA; Patri AK; McNeil SE
Investigation Publication Title	Characterization of nanoparticles for therapeutics
Investigation Publication Status	published
Investigation Publication Status Term Accession Number	
Investigation Publication Status Term Source REF	

Investigation Contacts

Example Investigation File Investigation Contacts Section

A	B
Investigation Person Last Name	Doe
Investigation Person First Name	John
Investigation Person Mid Initials	E
Investigation Person Email	doej@mail.nih.gov
Investigation Person Phone	1231231234
Investigation Person Fax	
Investigation Person Address	Laboratory Street, City, State 111111
Investigation Person Affiliation	Doe Laboratories
Investigation Person Roles	investigator
Investigation Person Roles Term Accession Number	
Investigation Person Roles Term Source REF	MO

Material

Example Investigation File Material Section

A	B
Material File Name	m_NCL-21.xls
Material Source Name	NCL-21

Study

Example Investigation File Study Section

A	B
Study Identifier	NCL200612A-CytoxicityLLC-PK1
Study Title	Cytotoxicity characterization in LLC-PK1 cells
Study Description	Nanoparticle biocompatibility was evaluated in the porcine renal proximal tubule cell line, LLC-PK1. Cytotoxicity was determined as described in the NCL protocol for LLC-PK1 Kidney Cytotoxicity Assay(GTA-1). Briefly, test materials were diluted to the desired assay concentrations in cell culture media. Cells were preincubated for 24 h prior to adding test material, reaching an approximate confluence of 80%. Cells were exposed to test material for 6, 24 and 48 h, and cytotoxicity was determined using the MTT cell viability and LDH membrane integrity assays.

Study Submission Date	2002-11-30
Study Public Release Date	2002-11-30
Study Disease	
Study Disease Term Accession Number	
Study Disease Term Source REF	
Study Outcome	NCL22, NCL23 and NCL24 were found to be minimally cytotoxic, under the testing conditions utilized.
Study File Name	s_cytotoxicity-LLC-PK1.xls

Study Design Descriptors

Example Investigation File Study Design Descriptors Section

A	B
Study Design Type	comparison
Study Design Type Term Accession Number	
Study Design Type Term Source REF	

Study Publications

Example Investigation File Study Publications Section

A	B
Study PubMed ID	18095846
Study Publication DOI	10.2217/17435889.2.6.789
Study Publication Author list	Hall JB; Dobrovolskaia MA; Patri AK; McNeil SE
Study Publication Title	Characterization of nanoparticles for therapeutics
Study Publication Status	published
Study Publication Status Term Accession Number	
Study Publication Status Term Source REF	

Study Factors

Example Investigation File Study Factors Section for Study Involving Biospecimens (such as in vitro, in vivo characterization). The study factor name and type must be of nanoparticle sample if the assay is applying a nanoparticle sample to a biological system.

A	B	C	D
Study Factor Name	nanoparticle sample	dose	time of exposure

Study Factor Type	nanoparticle sample	particle concentration	time of exposure
Study Factor Type Term Accession Number	NPO_1404	NPO_1830	NPO_1819
Study Factor Type Term Source REF	NPO	NPO	NPO

Example Investigation File Study Factors Section for Physico-Chemical Characterization Study. There should be no study factors of study factor type nanoparticle sample

A	B	C
Study Factor Name	temperature	solvent
Study Factor Type	temperature	solvent medium
Study Factor Type Term Accession Number	PATO_0000146	NPO_1855
Study Factor Type Term Source REF	PATO	NPO

Study Assays

Example Investigation File Study Assays Section

A	B	C
Study Assay Measurement Type	MTT Assay	LDH Release Assay
Study Assay Measurement Type Term Accession Number		NPO_1709
Study Assay Measurement Type Term Source REF		NPO
Study Assay Technology Type		
Study Assay Technology Type Term Accession Number		
Study Assay Technology Type Term Source REF		
Study Assay Technology Platform		
Study Assay Measurement Name	cell viability	LDH release
Study Assay Measurement Name Term Accession Number	NPO_1343	
Study Assay Measurement Name Term Source REF	NPO	
Study Assay File Name	a_MTT-LLCPK1.xls	a_LDH-LLCPK1.xls

Study Protocols

Example Investigation File Study Protocols Section

A	B	C	D	E	F	G	H	I	J
Study Protocol Name	Time-6-24-48 plate MTT assay	Test plate LDH assay	Time zero plate MTT assay	cell preparation in four 96-well plates	APAP positive control preparation	Triton-X100 positive control preparation	MTT assay reagent preparation	Test sample and positive control addition	LDH assay reagent preparation
Study Protocol Type				cell preparation	control preparation	control preparation	reagent preparation		reagent preparation
Study Protocol Type Term Accession Number									
Study Protocol Type Term Source REF									

Study Protocol Description	Test Plates: 6, 24 and 48 hour exposures (MTT Assay) 5.4.1 Remove appropriate test plate from incubator and replace media from Triton-X positive control wells (see plate format in Appendix) with 200 mL 1% Triton-X (made in Step 4.1.2). Let the plate set for 10 minutes at room temperature. Spin plate at 700 x g for 3 minutes. 5.4.2 Remove 100 mL of media from each well and transfer it to another plate, maintaining plate format. Use this plate immediately for the LDH assay (see Section 5.5). 5.4.3 Remove remaining media from original plate and discard. 5.4.4 Add 200 mL of fresh media to all wells. 5.4.5 Add 50 mL of MTT to all wells. 5.4.6 Cover in aluminum foil and incubate for 37°C for 4 hours. 5.4.7 Remove plate from incubator and spin at 700 x g for 3 minutes. 5.4.8 Remove media and MTT. 5.4.9 Add 200 mL of DMSO to each well. 5.4.10 Add 25 mL of glycine buffer to each well. Place on shaker to mix. 5.4.11 Read absorbance at 570 nm on plate reader using a reference wavelength of 680 nm.	Test Plates: 0, 6, 24 and 48 hour exposures (LDH Assay) Adapted from Biovision LDH Cytotoxicity Assay Kit, K311-400) 5.5.1 Add 100 mL of the Reaction Mixture (step 4.3.2) to each well of transfer plate. Shake plate on an orbital shaker briefly to mix samples. 5.5.2 Incubate at room temperature for up to 20 minutes in the dark. 5.5.3 Read the plate on plate reader at 490 nm using a reference wavelength of 680 nm.	5.2.1 Remove time zero plate from incubator and replace media from Triton-X positive control wells (see plate format in Appendix) with 200 mL 1% Triton-X (made in Step 4.1.2). Add 100 mL of media to the remaining wells. Let the plate set for 10 minutes at room temperature. Spin plate at 700 x g for 3 minutes. 5.2.2 Remove 100 mL of media from each well and transfer it to another plate, maintaining plate format. Use this plate immediately for the LDH assay (see Section 5.5). 5.2.3 Remove remaining media from original plate and discard. 5.2.4 Add 200 mL of fresh media to all wells. 5.2.5 Add 50 mL of MTT to all wells. 5.2.6 Cover in aluminum foil and incubate at 37°C for 4 hours. 5.2.7 Remove plate from incubator and spin at 700 x g for 3 minutes. 5.2.8 Aspirate media and MTT. 5.2.9 Add 200 mL of DMSO to all wells. 5.2.10 Add 25 mL of glycine buffer to all wells. Place on shaker to mix. 5.2.11 Read absorbance at 570 nm on plate reader.	5.1.1 Harvest cryopreserved cells from prepared flasks (limit to 20 passages). 5.1.2 Count cell concentration using a coulter counter or hemocytometer. 5.1.3 Dilute cells to a density of 2.5 x 105 cells/mL in M199 (3% FBS) cell culture media. 5.1.4 Plate 100 mL cells/well as per plate format (Appendix) for four 96-well plates (time zero, 6 hour sample exposure, 24 hour sample exposure, 48 hour sample exposure). The format indicates no cells in rows D and E as they serve as particle blanks to be subtracted from cell treatment wells. Each plate accommodates two samples (Rows A–C and F–H). Each nanoparticle is tested at nine dilutions. Column 11 receives the APAP positive control and column 12 receives Triton X-100. 5.1.5 Incubate plates for 24 hours at 5% CO2, 37°C and 95% humidity.	4.1.1 Acetaminophen (APAP) positive control: Add 19 mg to a total volume of 5 mL M199 Cell Culture Media (with 3% FBS) to make a 25 mM solution. Sterile filter using a 0.2 mm filter.	4.1.2 1% Triton-X-100 positive control: Add 1 mL of Triton-X-100 to 99 mL of media. Sterile filter using a 0.2 mm filter.	4.2.1 MTT solution: 5 mg/mL MTT in PBS, store for up to one month at 4°C in dark 4.2.2 Glycine Buffer: 0.1 M glycine (MW 75.07), 0.1 M NaCl (MW 58.44), pH 10.5, store at room temperature.	5.3.1 The highest concentration of nanoparticle tested should be at the limit of solubility. The test sample should be at physiological pH. Neutralization of acidic /basic test samples may be required. 5.3.2 Dilute test compound in media, making a total of 9 1: 4 dilutions. 5.3.3 Add 100 mL of each sample dilution and positive control to 6 hour, 24 hour and 48 hour exposure plates as per the plate format (Appendix).	4.3.1 Reconstitute catalyst in 1 mL dH20 for 10 min with occasional vortexing (stable for 2 weeks at 4°C). 4.3.2 Reaction mixture (for one 96-well plate): Add 250 mL of reconstituted catalyst solution to 11.25 mL of dye solution (stable for 2 weeks at 4°C).
Study Protocol URI	NCL_Method_GTA-1.pdf	NCL_Method_GTA-1.pdf	NCL_Method_GTA-1.pdf	NCL_Method_GTA-1.pdf	NCL_Method_GTA-1.pdf	NCL_Method_GTA-1.pdf	NCL_Method_GTA-1.pdf	NCL_Method_GTA-1.pdf	NCL_Method_GTA-1.pdf
Study Protocol Version	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Study Protocol Parameters Name									
Study Protocol Parameters Name Term Accession Number									
Study Protocol Parameters Name Term Source REF									

Study Protocol Components Name	MTT; acetaminophen; dimethyl sulfoxide; glycine; sodium chloride; triton-X-100; M199 cell culture media; fetal bovine serum; nanoparticle; costar 96 well flat bottom cell culture plates; plate reader; centrifuge set at 700-800 x g (Allegra X-15R, Beckman Coulter) with 96 well plate adapter; orbital plate shaker	acetaminophen; dimethyl sulfoxide; glycine; sodium chloride; triton-X-100; M199 cell culture media; fetal bovine serum; biovision LDH-cytoxicity assay kit; nanoparticle; costar 96 well flat bottom cell culture plates; plate reader; centrifuge set at 700-800 x g (Allegra X-15R, Beckman Coulter) with 96 well plate adapter; orbital plate shaker	MTT; acetaminophen; dimethyl sulfoxide; glycine; sodium chloride; triton-X-100; M199 cell culture media; fetal bovine serum; costar 96 well flat bottom cell culture plates; plate reader; centrifuge set at 700-800 x g (Allegra X-15R, Beckman Coulter) with 96 well plate adapter; orbital plate shaker	M199 cell culture media; fetal bovine serum; costar 96 well flat bottom cell culture plates; plate reader; centrifuge set at 700-800 x g (Allegra X-15R, Beckman Coulter) with 96 well plate adapter; orbital plate shaker	acetaminophen; M199 cell culture media; fetal bovine serum	triton-X-100; M199 cell culture media; fetal bovine serum	MTT; glycine; sodium chloride	acetaminophen; glycine; sodium chloride; triton-X-100; M199 cell culture media; fetal bovine serum; nanoparticle; costar 96 well flat bottom cell culture plates; plate reader; centrifuge set at 700-800 x g (Allegra X-15R, Beckman Coulter) with 96 well plate adapter; orbital plate shaker	biovision LDH-cytoxicity assay kit
Study Protocol Components Type	reagent; reagent; reagent; reagent; reagent; reagent; reagent; reagent; material; instrument; instrument; instrument	reagent; reagent; reagent; reagent; reagent; reagent; reagent; reagent; material; instrument; instrument; instrument	reagent; reagent; reagent; reagent; reagent; reagent; material; instrument; instrument; instrument	reagent; reagent;material; instrument; instrument; instrument	reagent; reagent; reagent	reagent; reagent; reagent	reagent; reagent; reagent	reagent; reagent; reagent; reagent; reagent; reagent; material; instrument; instrument; instrument	reagent
Study Protocol Components Type Term Accession Number	NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; ; NPO_1436; NPO_1436; NPO_1436	NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; ; NPO_1436; NPO_1436; NPO_1436	NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; NPO_290;NPO_290; ; NPO_1436; NPO_1436; NPO_1436	NPO_290; NPO_290; ; NPO_1436; NPO_1436; NPO_1436	NPO_290; NPO_290; NPO_290	NPO_290; NPO_290; NPO_290; NPO_290	NPO_290; NPO_290; NPO_290	NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; ; NPO_1436; NPO_1436; NPO_1436	NPO_290
Study Protocol Components Type Term Source REF	NPO; NPO; NPO; NPO; NPO; NPO; NPO; NPO; NPO; ; NPO; NPO; NPO	NPO; NPO; NPO; NPO; NPO; NPO; NPO; NPO; NPO; ; NPO; NPO; NPO	NPO; NPO; NPO; NPO; NPO; NPO; NPO; NPO; ; NPO; NPO; NPO	NPO; NPO; ; NPO; NPO; NPO	NPO; NPO; NPO	NPO; NPO; NPO	NPO; NPO ; NPO	NPO; NPO; NPO; NPO; NPO; NPO; NPO; ; NPO; NPO; NPO	NPO

Study Contacts

Example Investigation File Study Contacts Section

A	B
Study Person Last Name	Smith
Study Person First Name	Jane
Study Person Mid Initials	K
Study Person Email	smithj@mail.nih.gov
Study Person Phone	1231231235
Study Person Fax	
Study Person Address	Laboratory Street, City, State 111111
Study Person Affiliation	Doe Laboratories
Study Person Roles	investigator
Study Person Roles Term Accession Number	
Study Person Roles Term Source REF	MO