Assay File Examples

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The Assay File will vary depending on the type of assay performed and protocol and technology type leveraged. The following sections provide examples of Assay Files for common types of assays identified across nanotechnology resources. Assay File examples were created from assays and protocols performed by the Nanotechnology Characterization Laboratory (NCL) and identified in the NCL assay cascade.

Physico-Chemical Characterization Assays

Size Assay

Size by Dynamic Light Scattering (DLS) Assay

Dynamic Light Scattering (DLS) is a type of spectroscopy that uses a laser beam to irradiate a sample containing particles in suspension resulting in light scattering. Rapid fluctuations in scattering intensity around a mean value at a certain angle occur because of particle diffusion and are dependent upon on particle size. The calculated correlation function yields a diffusion coefficient, for a given temperature and viscosity, that can be used to calculate particle size.

Assay File	Assay Factors	Assay Measured Values	Supporting Information
Size by DLS	• Temperature • Solvent Medium	• Hydrodynamic Diameter • Polydispersity Index (PDI)	• Measuring the Size of Nanoparticles in Aqueous Solution using DLS • Characterization of Nanoparticles for Therapeutics • Dendrimer-Based MRI Contrast Agents

Zeta Potential Assay

Particle surface characteristics and charge play an important role in the particle's physical state, stability in different media, agglomeration tendencies, and interaction with biological systems. Zeta potential measurement provides an indirect measure of the net charge and as a tool to test batch-to-batch consistency.

Assay File	Assay Factors	Assay Measured Values	Supporting Information
Zeta Potential	• Concentration • Solvent Medium	• Zeta Potential	• Measuring Zeta Potential of Nanoparticles • Dendrimer-Based MRI Contrast Agents

In Vitro Characterization Assays

Cytotoxicity Assays

Hepatocarcinoma Cytotoxicity Assay (MTT and LDH)

The Hepatocarcinoma Cytotoxicity Assay tests the cytotoxicity of nanoparticle formulations in human hepatocarcinoma cells (Hep G2). The protocol utilizes two methods for estimation of cytotoxicity, 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) reduction and lactate dehydrogenase (LDH) release **(1, 2)**.

The MTT assay is a colorimetric assay that can assess the viability of cells by quantitation of the reduction of the yellow substrate MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to a product that has a purple color. This assay can measure the cytotoxicity of a chemical or drug by determining the affect of treatment on cell viability. The Assay File represents an example for a MTT cytoxicity assay (MTT) performed on three nanoparticle samples, where 9 dilutions of each nanoparticle sample are exposed to porcine proximal tubule cells for three different times of exposure (6h, 2h, and 48 h) (**3**).

LDH is a cytoplasmic enzyme that is released into the cytoplasm upon cell lysis. The LDH assay is a measure of membrane integrity. The basis of the LDH assay: (1) LDH oxidizes lactate to pyruvate, (2) Pyruvate reacts with the tetrazolium salt INT to form formazan, and (3) the water-soluble formazan dye is detected spectrophotometrically (**4,5**).

Assay File	Assay Factors	Assay Measured Values	Supporting Information
Hep G2 Hepatocarcinoma Cytotoxicity Assay (MTT)	• Time	• Cell Viability	• Hep G2 Hepatocarcinoma Cytotoxicity Assay Protocol • Characterization of Nanoparticles for Therapeutics • Dendrimer-Based MRI Contrast Agents
Hep G2 Hepatocarcinoma Cytotoxicity Assay (LDH)	• Time	• Cell Viability	• Hep G2 Hepatocarcinoma Cytotoxicity Assay Protocol • Characterization of Nanoparticles for Therapeutics • Dendrimer-Based MRI Contrast Agents

Caspase Apoptosis

Caspase Apoptosis assays monitor apoptosis by measuring the degree of the caspace (family of cysteine proteases) activity, an indicator of apoptosis. Caspase apoptosis assays measure the cleavage of the DEVD-AFC (Amino Tri-fluoromethyl Coumarin substrate to free AFC. Free AFC emits yellow-green fluorescence (**1,2,6**).

Assay File	Assay Factors	Assay Measured Values	Supporting Information
Caspase Apoptosis	• Concentration • Solvent Medium	• % Control Caspase Activity	• LLC-PK1 Kidney Cell Apotosis Assay • Functionalized Fullerenes NCL20071A

Blood Contact Assays

Hemolysis Assay

Hemolysis refers to the breaking open of red blood cells causing release of hemoglobin into the plasma. The Hemolysis assay measures the amount of hemoglobin in whole blood (TBH-Total Blood Hemoglobin) and the amount of hemoglobin released into plasma (PFH-Plasma Free Hemoglobin) when blood is exposed to nanoparticles. Hemoglobin is oxidized to methemoglobin and when reacting with cyanide results in Cyanmethemoblobin (CMH). The hemolysis assay can quantify the acute in vitro hemolytic properties of nanoparticles.

Assay File	Assay Factors	Assay Measured Values	Supporting Information
Hemolysis	• Concentration	• isHemolytic • Percent Hemolysis	• Analysis of Hemolytic Properties of Nanoparticles • Dendrimer-Based MRI Contrast Agents

In Vivo Characterization Assays

Disposition and Pharmacokinetics Assay

The Disposition and Pharmacokinetics assay is performed to determine the fate of a nanoparticle formulation in an organism, which can include its absorption, tissue distribution, metabolism or excretion (ADME). This is accomplished by detecting the whole formulation or its components at various body locations and time points after dosing. A variety of methods can be used to detect and quantify nanoparticle formulations and their components in vivo.

Assay File	Assay Factors	Assay Measured Values	Supporting Information
Pharmacokinetics	• Time	• Clearance at Time Zero (C0) • Area Under Curve (AUC) • Clearance (CL) • Half Life (T 1/2) • Volume of Distribution (V app)	• Distribution and Pharmcokinetics Description • Ceramide Liposomes NCL200702A
Tissue Distribution	• Time • Tissue	• Tissue Distribution	• Distribution and Pharmcokinetics Description • Ceramide Liposomes NCL200702A

References

1. ISO 10993-5 Biological evaluation of medical devices: Part 5 Tests for *in vitro* cytotoxicity.
2. F1903 – 98 Standard Practice for Testing for Biological Responses to Particles *in vitro*.
3. Alley, et al. (1988) *Cancer Res.* 48:589-601.
4. Decker, T. & Lohmann-Matthes, M.L. (1988) *J. Immunol Methods* 15:61-69.
5. Korzeniewski, C. & Callewaert, D.M. (1983) *J. Immunol Methods* 64:313-320.
6. Wang et al. (2005) Cell Bio. Int. 29: 489-496.