

Chemistry Specifications for Chemistry Services Contractors

National Toxicology Program

High Throughput Screen (HTS) Support

Final

August 11, 2016

## 1. *Introduction*

Requirements related to chemicals designated for general high throughput screen evaluations have been discussed in previous sections of these specifications. Requirements discussed in this section apply to sets of chemicals, test articles, and/or mixtures (chemicals) that are currently sent for evaluation in the high throughput screen program known as Tox21 run by the National Center for Advancing Translational Sciences (NCATS) at the National Institutes of Health.

Functional Activities in this section cover the procurement, handling and characterization of sets of chemicals for the Tox21 program. These activities are intended to apply to a set of chemicals, which constitute one complete plate used in the Tox21 program. Currently Tox21 plates consist of 1536 wells, containing 1408 individual chemicals or chemical mixtures. A typical set of chemicals addressed by the requirements in this section would therefore contain 1408 test articles (chemicals or chemical mixtures).

## 2. *HTS Chemical Procurement (HCP)*

1. The contractor shall identify sources and availability for multiple (up to 1500) chemicals required to complete a 1536-well plate for high throughput screen evaluation. Prior to procurement from a commercial source, the Contractor shall check the chemical inventory for each NTP chemistry support contract to determine if the chemical(s) is/are available from the other contract laboratories.
2. The contractor shall post the sourcing and quotation information to the NTP IMS as it is received.
3. As directed by the COR, the Contractor shall procure the necessary quantities (for costing purposes, assume < 10 g).
4. The contractor shall post the expected delivery date(s) for each chemical and the estimated time to receipt of all chemicals ordered, to the NTP IMS as soon as it is known.
5. Upon receipt of chemicals or test articles designated for HTS evaluation:
  1. The Contractor shall record the source and amounts received in the HTS chemical inventory system and assign chemical identification codes, including when necessary, NTP chemical identification numbers (CID)

and/or NTP substance identification numbers (SID) for each chemical received.

1. The Contractor shall be responsible for generating NTP and Tox21 CID and SID numbers for any HTS chemical not already listed in the Tox21 database (see: [http://www.epa.gov/ncct/dsstox/sdf\\_tox21s.html](http://www.epa.gov/ncct/dsstox/sdf_tox21s.html)). Refer to the EPA DSSTox website (<http://www.epa/ncct/dsstox>) for rules governing CID and SID number formats.
  2. Chemicals and/or test articles received, which are already listed in the Tox21 database, shall be assigned the NTP and Tox21 CID and SID numbers found in the Tox21 database when they are from the same source and have the same lot number as the listed chemical.
  3. Chemicals already in the Tox21 database, which are from a different source or have a different lot number, will receive the same NTP and Tox21 CID numbers, but will require new NTP and Tox21 SID numbers.
2. The Contractor shall examine the material and record the condition of the material, including the shipping container(s) and contents, and the shipped material (bulk chemical, test article, etc.) and the lot number(s).
  3. Photographs of the received material may be required in the event that the shipping container or sample container is found to be damaged or leaking. If photographs are taken, they shall be posted to the NTP IMS as soon as they are available.
6. The contractor shall report the work done in this functional activity following the reporting requirements given in Section 4. Reporting Requirements

### ***3. HTS Chemical Handling (HCH)***

1. As directed by the COR the Contractor shall perform some or all of the following activities on chemicals, test articles, and/or mixtures (chemicals) required to complete a 1536-well plate (typically 1408 chemicals or test articles) designated for high throughput screen evaluation.
  1. When a chemical or test article is received in multiple packages, homogenize the chemical.

2. Determine the solubility of each chemical at a concentration, typically 20 mM, in a solvent, typically DMSO (ACS spectrophotometric grade, Sigma-Aldrich Product No. 154938, or equivalent), specified by the COR.
  3. Prepare solutions, typically 50 mL at 20 mM or the maximum soluble concentration, of each chemical in a solvent designated by the COR, typically DMSO.
  4. Dispense small quantities (typically < 200 uL) of test article solutions into well-plates. Plates to be used typically have 96- or 384-wells. At the direction of the COR, multiple copies of each plate may be required.
2. The contractor shall create a plate map for each 96- or 384-well plate produced, which consists of a spreadsheet (Excel or equivalent) containing specific information about each chemical on the plate. An example plate map is given in Appendix 7.1.
  3. The Contractor shall create a structure file, which consists of a spreadsheet (Excel or equivalent) or structure data file (.sdf) containing specific chemical information, with specified field names for each chemical in the set, including but not limited to, CAS number, Tox21, DSSTox, and NTP substance and/or compound identification numbers, chemical structure, including, but not limited to, SMILES and InChi codes. An example structure file is given in Appendix 7.1. Definitions of the fields used in a structure file can be found on the DSSTox website: <http://www.epa.gov/ncct/dsstox>.
  4. The contractor shall examine the plated chemicals and record the condition of the plate(s) prior to shipment.
  5. The contractor shall report the work done in this functional activity following the reporting requirements given in Section 4. Reporting Requirements.
- 4. *HTS Identity and Purity Screen (HIPS)***
1. At the direction of the COR, the contractor shall conduct a screening analysis to determine the identity and estimate the purity of chemicals, test articles, and/or mixtures (chemicals) contained in a single 1536-well plate designated for high throughput screen evaluation. Typically a single plate contains 1408 individual chemicals.

1. These analyses shall consist of a single analytical system selected to unambiguously identify the chemical and estimate its purity simultaneously.
  2. The Contractor shall develop standard methods, selected to separate and identify the widest possible range of potential test articles. These systems, once developed shall not be optimized for individual chemicals. Typical analytical systems, which may be used for this purpose include the following:
    1. NMR (proton and carbon)
    2. Fourier Transform Infrared Spectrophotometry (FTIR)
    3. Inductively Coupled Plasma–Atomic Emission Spectrometry (ICP-AES) or ICP-MS (inorganic compounds)
    4. HPLC-MS, including UPLC
    5. GC-MS
  3. The Contractor shall employ the standardized system (GC-MS, LC-MS, etc.) selected to be the simplest and most cost-effective system that can successfully meet the analysis criteria for each chemical. If the selected system does not achieve acceptable identity and purity results, an alternate standardized system may be employed, with approval of the COR.
  4. When chromatographic methods are used, they shall employ temperature or mobile-phase gradients, which achieve adequate but not necessarily optimum separation of chemical components.
  5. Purity of chemicals selected for high throughput screen (HTS) evaluation must be  $\geq 95\%$  unless the COR approves a different target value.
2. The contractor shall report the work done in this functional activity following the reporting requirements given in Section 4. Reporting Requirements