Standard Operating Procedure:

Arylsulfatase A (ARSA) Enzyme Activity Assay Kit Cat. No. CS12

SOP ID:	SOPPCS12
Version Number:	V7.0
Version Date:	01 April 2026
Product Name:	ARSA Enzyme Activity Assay Kit
Matrices:	Dried Blood Spot

Authored By: Olive Lee White Bioanalytical Technician Enfanos, LLC.	Signed by Olive Lee White Olive Lee White I am the author of this document 01-Apr-26 | 12:52:27 PM PDT F8940F4C7FA34049A98114DD1A9A89B2	**Date:** 01-Apr-26
Reviewed By: Zackary M. Herbst Chief Science Officer Enfanos, LLC.	Signed by Zackary M. Herbst I have reviewed this document 01-Apr-26 | 6:16:20 PM PDT 95BFF6AAC0DB48458BA48EAB3EB25F25	**Date:** 01-Apr-26
Approved By: Hamid Khaledi Chief Executive Officer Enfanos, LLC.	Signed by Hamid Khaledi Hamid Khaledi I approve this document 01-Apr-26 | 12:52:37 PM PDT F28160B095964C7BBC2DB65A019F20F1	**Date:** 01-Apr-26

Table of Contents

Section 1. Materials and Reagents

Table 1.1: Reagents and Standards

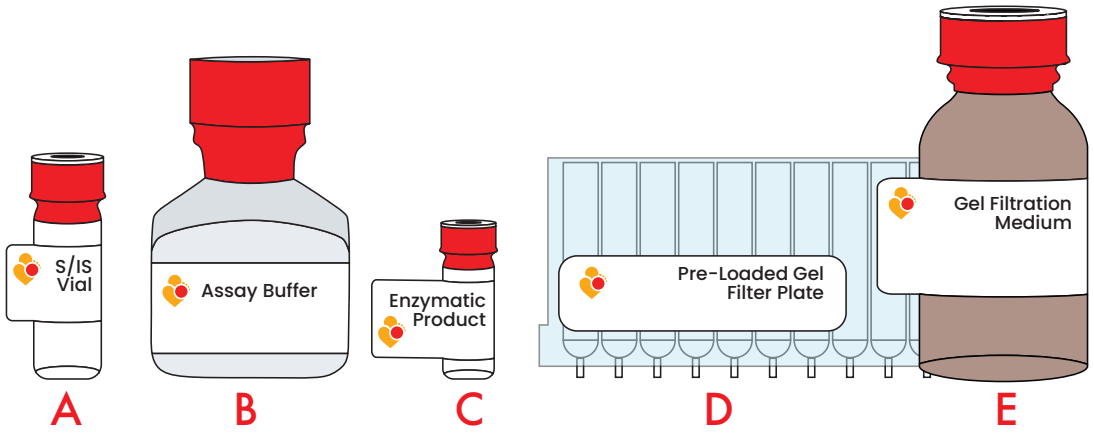
Material	Vendor	Vendor Reference
ARSA Enzyme Activity Assay Kit	Enfanos	CS12
MilliQ Purified Water (18.2 MΩ-cm resistivity), or Equivalent	Various	n/a
ACS Grade Ammonium Hydroxide (CAS: 1336-21-6)	Fisher	AA33285D9
Optima Grade Water (CAS: 7732-18-5)	Fisher	W6500
HPLC Grade Methanol (CAS: 67-56-1)	Fisher	A452

Table 1.2: Equipment and Consumables

Material	Vendor	Vendor Reference
MilliQ Water Purification System	MilliQ	Any System
3.2 mm (1/8") paper punch	Any	Any
Parafilm	Any	Any
45 µL MultisScreen Column Loader	Sigma Aldrich	MACL09645
Nunc 96-Well Fritted Filter plate (20 µm pore)	Fisher	278011
Deep-well 96-well plate (polypropylene)	Costar	3959
Silicone mat for 96-well plate	Costar	3080
(optional) 1.5 mL tube with snap cap (polypropylene)	VWR	89000-028
96-well microplate (polypropylene)*	Greiner Bio-One	651201
Zone-Free Sealing Film for 96-well microplate	Sigma	Z721646
Incubator with orbital shaker	Any	Any
Pipette, Single Channel, 2-20 µL (or equivalent)	Any	Any
Pipette, Single Channel, 100-1000 µL (or equivalent)	Any	Any
Pipette, Multichannel, 20-200 µL (or equivalent)	Any	Any
P10, P200, and P1000 Pipette tips	Any	Any
Calibrated Hamilton Syringes for accurate small volume measurement	Hamilton	Any
Centrifuge for 96-well plate (swinging bucket rotor)	Any	Any
2-8°C Refrigerator (Explosion Proof Recommended)	Any	Any
≤ -10°C Freezer	Any	Any
Fume Hood	Any	Any
Needle and Syringe (glass or polypropylene)	Any	Any

** Note: Choose autosampler vials or 96-well autosampler plates that are compatible with the UPLC autosampler that will be used for analysis.*

Section 2. Preparation of the Assay Cocktail



Scan to View the SDS

Figure 2.1 Components of the ARSA Enzyme Activity Assay Kit

2.1 Instructions for Preparation of the Assay Cocktail

The S/IS Vial(s) (A) and Assay Buffer (B) are provided by Enfanos as a part of the ARSA Enzyme Activity Assay Kit. Enzymatic Product (C) is provided as needed. The ARSA Enzyme Activity Assay Kit comes with either Pre-Loaded Gel Filter Plates (D) (Cat. No. CS12-PL) or a bottle of Gel Filtration Medium (E) (Cat. No. CS12-G).

Step 1. Allow the S/IS vial (A), Assay Buffer (B), and Pre-Loaded Gel Filter Plate (D) or Gel Filtration Medium (E) to warm to room temperature.

Step 2. Next, add 500 µL of the Assay Buffer (B) to the S/IS Mixture vial (for 50 assays) (A) using a calibrated Pipette. *For other vial sizes, reconstitution volume is printed on the S/IS Mixture Vial.*

Vortex the vial and allow to sit at room temperature for at least 30 minutes. After 30 minutes, vortex vial again and visually inspect with light behind it to ensure all

particulates and/or film on the glass is dissolved and a clear solution is formed. **A crystal clear solution must be obtained before you proceed**.

The resulting **Assay Cocktail** contains 450 µM ARSA-S and 1 µM ARSA-IS. If you have a question about the composition of the resulting **Assay Cocktail**, please contact us at contact@enfanos.com. Additional components (detergent, inhibitors, activators, buffer salt, etc.) are non-hazardous and are not listed.

Step 3. To prepare a 100 µM methanolic solution of the Enzymatic Product for LC-MS/MS tuning or standard curve preparation, add 10 µL of Methanol per 1 nmole of Enzymatic Product in the Enzymatic Product vial (C) using a Hamilton Syringe. Vortex to mix. Prepare additional dilutions as needed in 1:3 (v:v) Water:Methanol. The molecular weight of Enzymatic Product is 731.13 g/mol.

2.2 Storage and Stability of Reagents.

Formal stability data for long-term storage of S/IS Vials (A), Enzymatic Product Vials (C), Assay Buffer (B) are not available.

The S/IS vial (A) should be stored ≤ -10°C. The Assay Buffer (B) should be stored at 2-8°C. The Pre-Loaded Gel Filter Plate (D) and Gel Filtration Medium (E) should be stored dry at 4-30°C.

The **Assay Cocktail** (prepared in Section 2.1, Step 2) should be prepared fresh before use. Formal stability data for the **Assay Cocktail** is not available. Observed stability under internal testing suggests the **Assay Cocktail** is stable frozen at ≤ -10°C for at least 2 years and can be frozen and thawed before use at least 2 times.

The user is solely responsible for determining suitability of this method for any application.

Section 3. Recipes for Other Stocks and Buffers

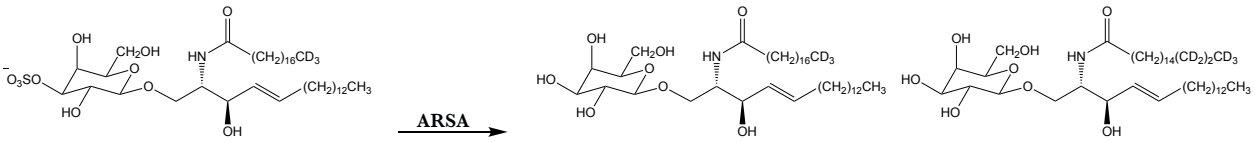
3.1 DBS Extraction Buffer (0.22-0.24% by wt. Ammonia in Assay Buffer)

To prepare 10 mL of DBS Extraction Buffer, combine 80 µL of Ammonium Hydroxide (28%-30% ammonia by weight, ACS Grade) and 9.92 mL of Assay Buffer in a 15 mL polypropylene tube. Prepare fresh before use.

** Note: Not all Ammonium Hydroxide solutions are 28-30% ammonia by weight. Read bottle label carefully and adjust the recipe as needed so the final solution contains 0.22-0.24% ammonia by weight.*

Section 4. Step-by-Step Method

Figure 4.1: Enzyme Assay Scheme



Exact Mass (Anion): 809.56
Molecular Weight (Anion): 810.18

ARSA Substrate

Exact Mass: 730.62
Molecular Weight: 731.13

ARSA Product

Exact Mass: 734.64
Molecular Weight: 735.15

ARSA Internal Standard

4.1 DBS Extraction

Step 1. Add one 3.2 mm DBS punch to a well in a 96-well deep-well plate (alternatively, use a 1.5 mL polypropylene microcentrifuge tube) and add 50 µL of the DBS Extraction Buffer (prepared in Section 3.1).

Seal the plate with a silicone 96-well plate mat (or cap the tube). Spin the plate (or tube) in a centrifuge at 200*g* for 10-20 seconds (sec) to bring all the liquid to the bottom, then incubate on an orbital shaker set to shake appropriately for 4 hours at room temperature.

** Note: The exact shaking setting will vary depending on the equipment used. The laboratory should determine the optimal shaking speed for their incubator/shaker as part of method implementation.*

4.2 Loading Gel Medium

Step 1. During the 4 hour DBS extraction, prepare the filtration plate. <u>If you received Pre-Loaded Gel Filter Plates (**D**) (Cat. No. CS12-PL), skip to Section 4.2, Step 4.</u>

Pour an excess amount of the Gel Filtration Medium (**E**) into the top of the MultiScreen Column Loader over a sheet of aluminum foil. The foil will collect the extra medium for re-use. Prepare more wells than needed in case any samples need to be re-filtered (see Section 4.4, Step 2 and Annex 2.3 "Second Filtration of DBS Extracts").

** Note: Tape off wells you don't want to fill by using paper adhesive tape. Remember that the first column of the black MultiScreen Column Loader starts with H1 on the top left and ends with A1 on the bottom left (inverse of the final plate). See Figure 4.2.*

Step 2. Using the provided scraper, push the material into the column loader wells and check that they are packed to the top with resin. Scrape off excess medium onto the aluminum foil and return it to the bottle for re-use.

If any wells were taped off, remove the tape before sliding the empty, clean Nunc Deep-Well Fritted Filter Plate into place, upside-down, onto the MultiScreen Column Loader, so the top of the plate is resting on the top of the loader. Be certain to push the plate up against the end stop of the loader so all the wells are aligned (*See Figure 4.2*).

Step 3. Squeeze the plate and loader together and turn the entire assembly over so the filter plate is now right-side-up (see Figure 4.2). Set the assembly down on the bench with the loader and filter plate still together. Tap the loader plate gently with your fingers to ensure all resin has fallen to the wells of the filter plate. Remove the loader from the top of the filter plate and assure no particles remain.

Step 4. With a Pipette, add 300 µL of MilliQ Water to each well with dry resin.

Seal the plate using zone-free sealing film to minimize evaporation of water from the wells and leave the plate on the bench at room temperature for 3 hours.

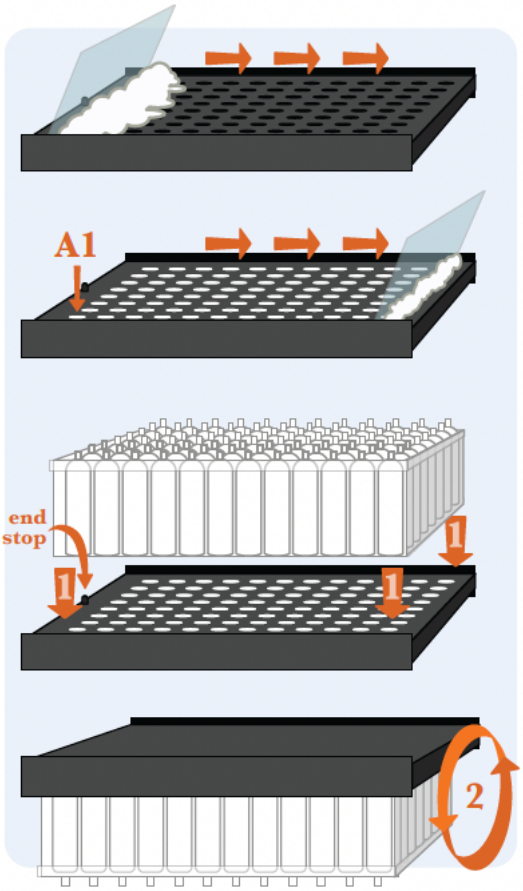


Figure 4.2 MultiScreen Column Loader Plate Stacking Guide

4.3 Condition Filter Plate

Step 1. Pour an aliquot of Assay Buffer (**B**) into a multichannel Pipette solvent reservoir and allow it to warm to room temperature.

Step 2. Just before use, remove the zone-free sealing film from the filter plate and place the filter plate on top of a 0.5 mL 96-well plate (collection plate) to collect the liquid which elutes from the filter plate.

Stack the two plates in a centrifuge and spin the plates at 800*g* for 1 minute. Discard the liquid from the 96-well collection plate after centrifugation and replace the plate to collect more liquid in the next Step.

** Note: If centrifuging only 1 plate, use an empty filter plate and reservoir plate to balance the empty side of the centrifuge rotor.*

Step 3. Condition the gel medium three more times by adding 200 µL of room temperature Assay Buffer (**B**) to each well with a Pipette. Centrifuge after addition of each 200 µL aliquot at 800g for 1 minute. Discard the liquid after each centrifugation step to prevent wells from becoming full.

Step 4. After conditioning the filter plate, place a clean 0.5 mL 96-well plate (receiver plate) under it and set the two plates on the bench.

4.4 Filter DBS Extract

Step 1. After DBS incubation, spin the DBS plate in a centrifuge at 200g for 10-20 seconds to bring all the liquid to the bottom, then transfer 30 µL of the extract to a well in the filter plate containing resin. Add the DBS extract dropwise (see Annex 2.2, "Dropwise Addition") to the center of the resin in each well, then add 15 µL of room temperature Assay Buffer (**B**). Cover the plate stack with the silicone 96-well plate mat and spin in a centrifuge at 800*g* for 1 minute.

** Note: The exact centrifugal g-force will vary depending on the equipment used. The laboratory should determine the optimal g-force for their centrifuge as part of method implementation. 800g is the recommended starting point for determination (see Annex 2.4, "Centrifugation").*

Step 2. Under bright lights, place the receiver plate on a piece of white paper. Inspect each well carefully to determine if any have a slight yellow color.

** Note: Yellow color indicates that too much material has eluted, and some small molecular weight fraction of the blood extract may have also eluted. This may result in ARSA inhibition. If yellow color is found, a second filtration is required. In this case, completely transfer the liquid from the yellow wells into the extra wells of conditioned resin that were previously prepared in the filter plate (see Section 4.2, Step 1; see Annex 2.3 "Second Filtration of DBS Extracts" for tips), do not add any additional aliquots of the Assay Buffer (B). Spin as above to collect the eluate.*

4.5 Enzyme Incubation and Quenching

Step 1. To each well in the receiver plate, add 10 µL of **Assay Cocktail**. Seal the plate with a silicone 96-well plate mat (or cap the tube). Spin the plate (or tube) in a centrifuge at 200*g* for 10-20 seconds (sec) to bring all the liquid to the bottom, then incubate on an orbital shaker set to shake appropriately for 16 hours at 37°C. Record the time when the plate is put on the incubator. This is the start time for the enzyme activity assay incubation.

Step 2. After incubation, spin the plate (or tube) in a centrifuge at 200g for 10-20 seconds to bring all the liquid to the bottom, then quench the reaction by addition of 300 µL of Methanol. Pipet the mixture up and down 10 times to mix well.

Record the time when the Methanol is added: this is the end time for the enzyme activity assay incubation.

Cover the plate with the silicone 96-well plate mat (or cap the tube) and spin in a centrifuge at 3000*g* for 5 minutes at ambient temperature to pellet any particles.

Step 3. After centrifugation, avoiding the particulate pellet at the bottom of the well, transfer 150 µL of the supernatant to a 96-well polypropylene microplate appropriate for the autosampler you will use.

** Note: If the plate must be stored overnight prior to LC-MS/MS analysis, cover with zone-free sealing film and store at ≤ -10°C. On the day of LC-MS/MS analysis, allow the plate to warm to room temperature and proceed to Step 4.*

Step 4. On the same day as LC-MS/MS analysis, add 50 µL of Optima Grade Water to each well. Mix with a multichannel Pipette by pipetting up and down 10 times.

Seal the plate using zone-free sealing film, or as appropriate for your autosampler, and submit to LC-MS/MS analysis (see Section 5 for recommended method conditions).

Section 5. Recommended Liquid Chromatography Tandem-Mass Spectrometry (LC-MS/MS) Method

Table 5.1: Materials and Reagents

Material	Vendor	Vendor Reference
Optima Water (LC-MS) (CAS: 7732-18-5)	Fisher	W6500
Optima Acetonitrile (LC-MS) (CAS: 75-05-8)	Fisher	A955
Optima Isopropanol (LC-MS) (CAS: 67-63-0)	Fisher	A461
Optima Formic Acid (LC-MS) (CAS: 64-18-6)	Fisher	A117
CSH FluoroPhenyl Column, 1.7 µm, 2.1 X 50 mm*	Waters	186005296
CSH FluoroPhenyl Pre-column, 1.7 µm, 2.1 X 5 mm*	Waters	186005303
Acquity 2D UPLC System*	Waters	Or Equivalent
Triple Quad Mass Spectrometer	Waters	Or Equivalent

** Note: While Enfanos recommends use of Waters equipment for LC-MS/MS analysis, equivalent instrumentation may be used for analysis of our enzyme activity assay products and internal standards.*

5.1 LC Method.

- **Solvent A.** 0.1% Formic Acid in 50:50 (v:v) Water:Acetonitrile
- **Solvent B.** 0.1% Formic Acid in 50:50 (v:v) Isopropanol:Acetonitrile
- **Weak Needle Wash.** 25:25:50 (v:v:v) Methanol:Isopropanol:Water
- **Strong Needle Wash.** 47.5:47.5:5 (v:v:v) Methanol:Isopropanol:Water
- **Column Temperature.** 30°C

Table 5.2: Gradient Program.

Time (min)	Flow (mL/min)	Solvent B (%)
initial	0.7	0.0
0.50	0.7	0.0
1.00	0.7	90
1.50	0.7	100
1.95	0.7	100
2.00	0.7	0.0

** Note: To minimize contamination of the ESI source, we advise to divert the LC flow to waste except in the retention time region (see Table 5.3) where the product and internal standard elutes (~1.18 min).*

5.2 MRM Method.

MRM Transitions (see Table 5.3) are measured in ESI positive mode.

** Note: Method developed for analysis on Waters XEVO TQ Instrument (or instrument with equivalent sensitivity). If a higher sensitivity instrument, such as a Waters XEVO TQ-S Micro is being used we recommend adjusting sample preparation so the injected solution contains less moles of analyte or detuning the collision energy of your analytes so the internal standard peak areas are approximately 100,000 AUC. This will ensure linearity throughout the analytical range.*

Table 5.3: MRM Method.

Analyte	Parent (m/z)	Product (m/z)	Retention Time (min)
ARSA Product	731.8	264.4	1.18
ARSA Internal Standard	735.9	264.4	1.18
ARSA Substrate	811.5	264.4	1.36

** Note: Transitions, cone voltage, collision energy, and retention times need to be confirmed or determined by tuning on your LC-MS/MS instrument in the usual way.*

5.3 Formula for Specific Activity.

$$\boldsymbol{ARSA\ Specific\ Activity\ (\mu mol/h/L)} = \frac{\boldsymbol{P}}{\boldsymbol{IS}} \times \frac{\boldsymbol{0.00001\ \mu mole}}{\boldsymbol{16\ h\ \times 0.6 \times 3.2 \times 10^{-6} L}}$$

To obtain the enzymatic activity, multiply the product-to-internal standard peak area ratio by the micromoles of internal standard in the assay (0.00001 µmol). This number is then divided by the incubation time (16 hours) and 3/5th of the liters of blood in a 3.2 mm DBS punch (0.6 x 3.2 x 10^{-6} L) as determined by Adam et. al., 2000.

** Note: To calculate the incubation time, subtract the enzyme activity assay end time (see Section 4, Step 2) from the enzyme activity assay start time (see Section 4, Step 1).*

*** Note: This procedure describes the preparation and handling of research-use-only materials. It does not establish or support clinical testing, diagnostic use, or patient result interpretation. Any use of this product in laboratory developed tests or clinical applications is the sole responsibility of the user. Enfanos does not validate or support clinical or diagnostic use.*

Section 6. References

References are provided for general scientific background only and do not imply clinical performance, intended use, or validation of this method.

Adam, B.W., Alexander, J.R., Smith, S.J., Chace, D.H., Loeber, J.G., Elvers, L. and Hannon, W.H., 2000. Recoveries of phenylalanine from two sets of dried-blood-spot reference materials: prediction from hematocrit, spot volume, and paper matrix. *Clinical Chemistry*, 46(1), pp.126-128.

Hong, X., Daiker, J., Sadilek, M., Ruiz-Schultz, N., Kumar, A.B., Norcross, S., Dansithong, W., Suhr, T., Escolar, M.L., Scott, C.R. and Rohrwasser, A., 2021. Toward newborn screening of metachromatic leukodystrophy: results from analysis of over 27,000 newborn dried blood spots. *Genetics in Medicine*, 23(3), pp.555-561.

Section 7. Document Audit Trail

Previous versions of this SOP are controlled internally by Enfanos, LLC and are not distributed. A high-level summary of changes between versions is provided upon request. The summary is intended for informational purposes only and does not constitute instructions for use or validation guidance.

This document is not intended to support regulatory submissions or clinical validation.

Annex 1: Assay Buffer Recipe

Assay Buffer is provided as part of the ARSA Enzyme Activity Assay Kit (Cat. No. CS12). In the case that extra Assay Buffer is required, the recipe is given below.

Table A1.1: Assay Buffer Materials and Reagents

Material	Vendor	Vendor Reference
MilliQ Purified Water (18.2 MΩ-cm resistivity), or Equivalent	Various	n/a
Sodium Acetate, Anhydrous (ACS Grade)	Sigma Aldrich	241245
Glacial Acetic Acid (ACS Grade)	Fisher	AA36289K3
Sodium Taurodeoxycholate hydrate ("NaTDC")	Carbosynth	FS45995

A1.1 Solution A: (80 mM Sodium Acetate, 2 g/L NaTDC in Water)

** Note: Prepare 10 % more than what is needed for the Solution A (below)*

To prepare 1 L of Solution A, combine 6.56 g of Anhydrous Sodium Acetate (ACS Grade; 82.03 g/mol) and 2.0 g of Sodium Taurodeoxycholate (NaTDC) in 1.0 L of purified water (MilliQ water, or equivalent). Stir until powder has fully dissolved. Store remaining solution in a borosilicate glass storage bottle at 2-8°C for up to 6 months.

A1.2 Solution B: (80 mM Acetic Acid, 2 g/L NaTDC in Water)

** Note: Prepare 10 % more than what is needed for the Solution B (below)*

To prepare 1 L of Solution B, combine 4.58 mL of glacial acetic acid (ACS Grade; 60.05 g/mol, 1.049 g/mL) and 2.0 g of Sodium Taurodeoxycholate (NaTDC) in 995.42 mL of purified water (MilliQ water, or equivalent). Stir until powder has fully dissolved. Store remaining solution in a borosilicate glass storage bottle at 2-8°C for up to 6 months.

A1.3 Recipe for Assay Buffer

Calibrate your pH meter using, at minimum, a 2-point calibration with pH 4 and 7 buffers. To prepare 1 L of Assay Buffer, combine 400 mL of Solution A and 600 mL of Solution B in a borosilicate glass storage bottle and mix well. Adjust the pH (to higher pH by addition of Solution A or lower pH by addition of Solution B) until the final pH of 4.5 is achieved. Note that the final volume may exceed 1L. Store remaining solution in a borosilicate glass storage bottle at 2-8°C for up to 6 months.

Annex 2: Tips for Laboratories

Some tips are given below to avoid user error, account for method variation, and to guide decisions to be made by individual laboratories.

A2.1 Calculation of ARSA Activity

On Page 15 of this SOP, we give the formula to calculate ARSA activity in the units of µmol/h/L. When QCs are provided from Enfanos, LLC, we recommend switching to reporting of ARSA activity as % of the QC High. This is done by calculating the average activity of the QC High replicates and dividing each sample activity by the QC High average activity.

A2.2 Dropwise Addition

Dropwise addition of DBS extracts (Section 4.4, Step 1 on Page 11) should be done as follows:

- Be certain to use the appropriate size of Pipette for slow dispensing of small volumes. For example, dispensing of 15 µL should be done with a Pipette with a range of 2-20 µL (not 10-200 µL);
- Hold the Pipette vertically so the tip is about 1 cm above the top of the gel pad;
- Add liquid slowly to avoid forming a stream or jet;
- Ensure the liquid leaves the tip dropwise and falls onto the gel bed rather than the side walls of the well;
- Use the shallow-well filter plate referenced in this SOP (Nunc 96-Well Fritted Filter Plate, Cat. No. 278011) so that it is easier to see down inside the well.

A2.3 Second Filtration of DBS Extracts

After the first filtration of the 30 µL of DBS extracts (see Section 4.4 on Pages 11-12), some labs are routinely getting zero yellowish wells, while some labs are getting about 10% yellowish wells. For the latter labs, it may be easier to run all wells through a second filter plate with conditioned gel filtration medium (see Section 4.2, Step 1 for preparation of the gel filter plate) rather than re-running only a few wells. It is up to each individual lab to decide how to handle this.

Remember, if you run a second gel filtration step you must also re-filter the QC Low and the QC High (2 filtration steps total) since some ARSA may be lost to the gel each time you run the gel filtration (ARSA activity will tend to drop if you run 2 gel filtration steps). This is another reason why ARSA activity is reported as a percentage of the QC High.

In the case of two gel filtration steps, the 100% value will be the ARSA activity of the QC High that has been submitted to two rounds of gel filtration.

A2.4 Centrifugation

The centrifuge speed is important. When it is indicated to use 800g, do not instead use 800 rpm. Note that 800g is the same as 800 rcf, but this does not equal 800 rpm. Reference the g-force chart for your rotor to ensure that 800g is being used.

Based on your make and model of centrifuge, you may desire to optimize the centrifugation conditions during Section 4.4 of the Procedure. If you are consistently getting yellow or red eluate during Step 1, you may reduce the centrifuge speed until your eluates are no longer yellow/red. Note that the obtained enzyme activity is dependent on the speed of centrifugation. While our lab uses 800g, adjusting to a speed that is too high will lead to red eluates while too low of a speed may lead to reduced enzyme activity.

When using 1 minute for a spin, note that 1 minute starts when the motor reaches the final speed.

Annex 3: Recipes for LC Solvents

A3.1 Solvent A: (0.1% Formic Acid in 50:50 (v:v) Water:Acetonitrile)

To prepare 1 L of Solvent A, combine 500 mL of Optima Water (LC-MS) and 500 mL of Optima Acetonitrile (LC-MS) using a 500 mL or 1 L graduated cylinder in a 1 L glass solvent bottle. Measure each solvent separately in the graduated cylinder and combine them only in the 1 L glass solvent bottle to avoid the partial molar volume effect. Add 1.0 mL of Optima Formic Acid (using a P1000 Pipette). Stir until the material is mixed and degas. Store remaining solution at room temperature for up to 6 months.

A3.2 Solvent B: (0.1% Formic Acid in 50:50 (v:v) Isopropanol:Acetonitrile)

To prepare 1 L of Solvent B, combine 500 mL of Optima Isopropanol and 500 mL of Optima Acetonitrile (LC-MS) using a 500 mL or 1 L graduated cylinder in a 1 L glass solvent bottle. Measure each solvent separately in the graduated cylinder and combine them only in the 1 L glass solvent bottle to avoid the partial molar volume effect. Add 1.0 mL of Optima Formic Acid (using a P1000 Pipette). Stir until the material is mixed and degas. Store remaining solution at room temperature for up to 6 months.

A3.3 Weak Needle Wash: (25:25:50 (v:v:v) Methanol:Isopropanol:Water)

To prepare 1 L of Weak Needle Wash, combine 500 mL of Optima Water (LC-MS), 250 mL of Optima Methanol (LC-MS), and 250 mL of Optima Isopropanol using a 500 mL or 1 L graduated cylinder in a 1 L glass solvent bottle. Measure each solvent separately in the graduated cylinder and combine them only in the 1 L glass solvent bottle to avoid the partial molar volume effect. Stir until the material is mixed and degas. Store remaining solution at room temperature for up to 6 months.

A3.4 Strong Needle Wash: (47.5:47.5:5 (v:v:v) Methanol:Isopropanol:Water)

To prepare 1 L of Strong Needle Wash, combine 475 mL of Optima Methanol (LC-MS) and 475 mL of Optima Isopropanol using a 500 mL or 1 L graduated cylinder and 50 mL of Optima Water (LC-MS) using a 100 mL graduated cylinder in a 1 L glass solvent bottle. Measure each solvent separately in the graduated cylinders and combine them only in the 1 L glass solvent bottle to avoid the partial molar volume effect. Stir until the material is mixed and degas. Store remaining solution at room temperature for up to 6 months.