

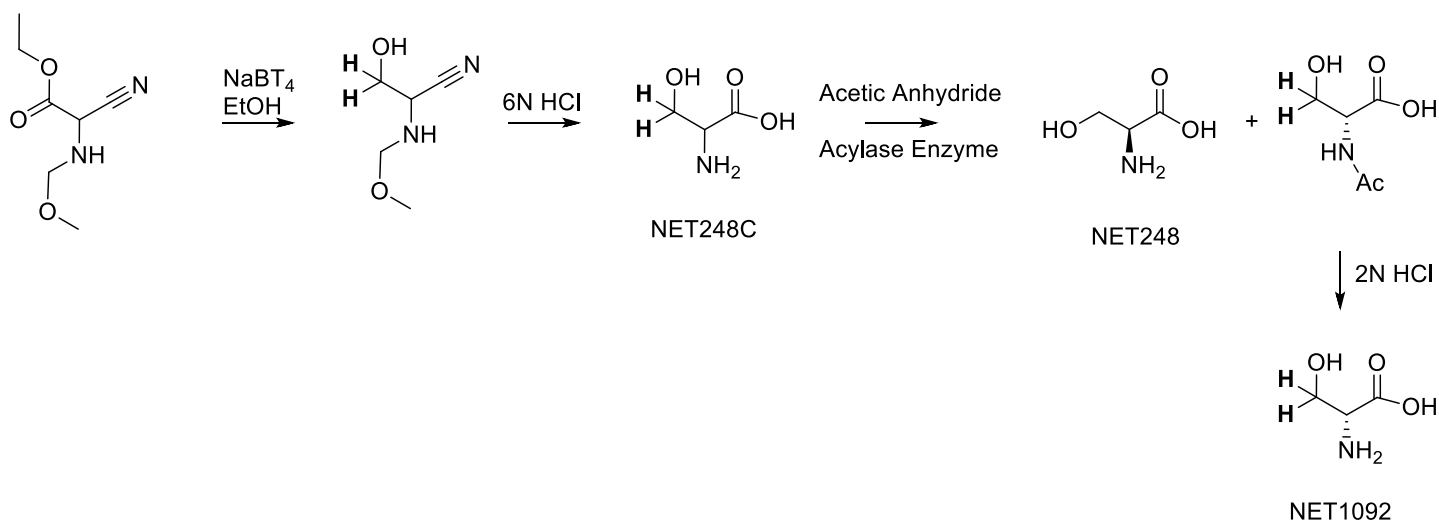
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Document Title: Synthesis & Purification NET248			Version: 06	
			Document Status: Released	Change Number: 120-RSP00119
Document Responsibility: Reagent Manufacturing		Periodic Review Date: 12 December 2020		Effective Date: 12 December 2015
Functional Areas Impacted: 140 - Reagent Manufacturing		Sites Impacted: 120 - Boston		

Function	Full Name	User ID	Status	Date	Time
Quality Assurance (R)	Marisa Egan	EGANMN	Review Completed	08 December 2015	11:20:53
Reagent manufacturing (R)	Subbareddy Gaddam	GADDAMSN	Review Completed	08 December 2015	11:11:20
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Revisions: 1. In HPLC Post Purification notes, add stock solvent 2% ethanol in water.	Reasons: 1. Increase clarity, lab bulk 50% Ethanol, stock bulk 2% ethanol.
Prior Revisions: 5 1. Add reaction schematic and refer to NET1092, D-Serine document for D,L separation procedure.	Reasons: 1. For more complete information.
Prior Revision: 4 1. Updated preparative chromatography conditions.	Reasons: 1. To reflect current best practices.
Prior Revision: 3 1. Add 'most updated' to Individual Lot Record section and remove 1-04-40-RSP-609.	
Prior Revision: 2 1. Review and update document number from 018795-T248. 2. Add [3H] Reaction Conditions.	
Prior Revision: 1 1. Update hand-written changes & new format.	



GENERAL COMMENTS:

Refer to synthesis guide 1-04-40-RSP-T1092, D-[³H(G)]-Serine, for detailed instructions to prepare acetylated serine, isolate the L-[³H(G)]-Serine, and save the D-[³H(G)]-acetyl serine for further purification of NET1092 as needed.

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Catalog #: NET248

Compound name: Serine, L-[³H(G)]-

Consult the lab's compound files for detailed information. Immediately notify the lab supervisor of problems, unusual occurrences, or questions.

SAFETY:

Required Gloves: VINYL, NITRILE OR LATEX

PREPARATIVE CHROMATOGRAPHY:

Column:	Poly LC Hydroxyethyl A (25 X 0.9 cm)
Injection solvent:	< 10 ml 20%A / 80%B.
UV	214 nm
Approximate retention time:	~20min @ 3mL/min, ~47 min @2mL/min
Solvent A	Water
Solvent B	1% TEAA (pH 4.0) *This is the strong solvent*
Solvent C	Acetonitrile
Solvent D	Methanol

Step	Time	Flow	Solvent A	Solvent B	Solvent C	Solvent D	Curve
0	20	3.0	0	30	70	0.0	0.0
1	60	3.0	0	40	60	0.0	1.0
2	40	0.1	0	40	60	0.0	0.0

POST PURIFICATION:

Reconstitute in **50% Ethanol** at 10mCi/ml, remove 100 ul sample for mass spec if needed, then dilute to approximately 2 to 8 mCi/ml in **50% Ethanol** for lab bulk storage.

Stock Bulk concentration is 1mCi/mL **2% Ethanol**.

Assay Solvent: Water

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ANALYTICAL METHODS

Stock Specification

Minimum initial purity: 97%

Out-of-stock purity: 90%

Required Method

Reverse phase HPLC analysis:

Column:	SCX analytical column
UV	214 nm
Approximate retention time:	~7 - 11 minutes
Solvent A	Water
Solvent B	2-5 mM KHPO ₄ , pH2.5
Solvent C	Acetonitrile
Solvent D	Methanol
Molarity of KHPO ₄ may be adjusted to get retention time greater than 7 minutes, depending on condition of SCX column	

Step	Time	Flow	Solvent A	Solvent B	Solvent C	Solvent D	Curve
0	0.5	1.0	0	100	0	0	0
1	19.9	1.0	0	100	0	0	0
2	0.1	1.0	0	100	0	0	0

Optional, Chiral purity

Column:	Crownpak (+)
UV	214 nm
Approximate retention time: L-Serine	~5.5 minutes
Approximate retention time: D-Serine	~4.3 minutes
Solvent A	Water
Solvent B	Perchloric acid
Solvent C	Acetonitrile
Solvent D	Methanol

Step	Time	Flow	Solvent A	Solvent B	Solvent C	Solvent D	Curve
0	0.5	0.4	0	100	0	0	0
1	19.9	0.4	0	100	0	0	0
2	0.1	0.4	0	100	0	0	0

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SPECIFIC ACTIVITY

Stock Specification

Specific Activity Acceptable Range: 15 to 40 Ci/mmol

Method: Mass Spec.

Molecular Formula: C₃H₇NO₃

Molecular Weight: 105

FINAL STOCKING DATA

Stock Specification:

Concentration:

(Range: -3% to +5%)

Solvent for Assay: water

Stock Bulk

Stock Concentration:	1.0 mCi/mL
Stock Solvent:	Water/Ethanol (98:2)
Solvent Density:	0.996
Stock Vessel:	Tared bulk bottle
Stock Storage Conditions:	Refrigerator

Lab Bulk

Bulk Concentration:	~2 to 8 mCi/mL
Bulk Solvent:	Ethanol/Water (1:1).
Solvent Density:	0.88
Bulk Vessel:	Tared bulk bottle
Bulk Storage Conditions:	Freezer

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Line

No. [3H] Reaction Conditions

- 1 Cat No: NET248C
- 2 Compound Name: D/L-SERINE, [3H(G)]-
- 3 Operation: SODIUM BOROHYDRIDE (NET023X)
- 4 *Precursor: ETHYLACETAMIDOCYANOACETATE (MW=170.21)
- 5 Precursor Amount: 68 mg
- 6 Solvent: 2.5 ml ETHANOL
- 7 Co-Reagent: NONE
- 8 Hot Reagent: 20 mg SODIUM BOROTRITIDE 023X
- 9 Scale Ratio, moles Hot / moles Precursor: N/A
- 10 Reaction Temperature: 25 DEGREES C
- 11 Stop Reaction After: 1.5 HOURS, then **reflux for 24 hours.
- 12 Quenching Solvent: 1 ml GLACIAL ACETIC ACID
- 13 Labile Solvent: 2 x 2 ml METHANOL
- 14 Filtration Solvent: 3 x 3 ml METHANOL
- 15 Packaging Solvent: 50 ml 50% ethanol.

Comments or Special Instructions:

1. Chill the NET023X in an ice bath.
2. *Dissolve precursor in 2.5 ml ethanol
3. Slowly inject the precursor (dropwise), into the NET023X.
4. **After filtration, reflux for 24 hours in 5 ml of 6 N HCL.
5. Remove labiles & package.