JUL 05, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.kxygx3exzg8j/v1

Protocol Citation: alow 2023. Preparation of Artificial Urine. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kxygx3exzg8j/v1

Protocol status: Working
We use this protocol and it's working

Created: Jul 05, 2023

Last Modified: Jul 05, 2023

PROTOCOL integer ID: 84532

Preparation of Artificial Urine

alow[1]

[1]University of Edinburgh

alow alow

ABSTRACT

A method for producing artificial urine as a media for bacterial experiments

MATERIALS

	A	B	C	D	E	F
	Formula	Name	Mass (g) per 100 mL	CAS number	Catalogue code	Manufacturer
	Na2SO4	Sodium sulphate	0.17			
	Na3C6H5O7.2H2O	Tri-sodium citrate	0.072			
	C4H7N3O	Creatinine	0.081	60-27-5	K5225680	Sigma
	CH4N2O	Urea	1.5	57-13-6	15604-1Kg	Sigma
	KCl	Potassium chloride	0.2308			
	NaCl	Sodium chloride	0.1756			
	CaCl2	Calcium chloride anhydrous	0.0185	10043-52-4	C1016-500G	Sigma

	A	B	C	D	E	F
	NH4Cl	Ammonium chloride	0.1266			
	K2C2O4.H2O	Potassium oxalate	0.0035	607-007-00-3	G0425-250G	Honeywell
	MgSO4.7H2O	Magnesium sulphate	0.1082	10034-99-8	M2773-500G	Sigma
	NaH2PO4.2H2O	Sodium dihydrogen orthophosphate dihydrate	0.2912	13472-35-0		
	Na2HPO4.2H2O	Sodium phosphate monobasic dihydrate	0.0831	10028-24-7	71645-1Kg	Sigma
		Bacteriological Peptone	0.2		LP0037	Oxoid
		Yeast Extract	0.0018		70161-100g	Fluka
		Ultrapure water				

Table 1: Reagents required

1 Take twelve 50 mL tubes and label with sufficient information. There are 12 reagents (Table 1 in Materials) which require plastic tube storage so you can label these 1 to 12 but missing out

number 4 for Urea. You will need two tubes for reagent 9, Potassium oxalate.

2 Weight out the chemical powders to the labelled tubes as described in table 2.

	A	B	C	D
	Reagent ID	Formula	Name	Recommended Stock Preparation
	1	Na2SO4	Sodium sulphate	8.5g in 50mL water (X100)
	2	Na3C6H5O7.2H2O	Tri-sodium citrate	3.6g in 50mL water (X100)
	3	C4H7N3O	Creatinine	4.05g in 50mL water (X100) do not chill
	4	CH4N2O	Urea	(15g per 100mL) 22.5g in 150mL water (X10) made fresh on the day of combining reagents. Endothermic, warm to 37C to prevent cooling the mixture upon addition and causing other salts to precipitate Calculate how much required on the day.
	5	KCl	Potassium chloride	11.54g in 50mL water (X100)
	6	NaCl	Sodium chloride	8.78g in 50mL water (X100)
	7	CaCl2	Calcium chloride anhydrous	0.925g in 50mL water (X100) caution - alkali
	8	NH4Cl	Ammonium chloride	6.33g in 50mL water (X100)
	9	K2C2O4.H2O	Potassium oxalate	2 steps: dissolve 1.75g in 50mL water (X1000) then transfer 5mL to a new tube and make up to 50mL with water for X100
	10	MgSO4.7H2O	Magnesium sulphate	5.41g in 50mL water (X100)
	11	NaH2PO4.2H2O	Sodium dihydrogen orthophosphate dihydrate	14.56g in 50mL water (X100)
	12	Na2HPO4.2H2O	Sodium phosphate monobasic dihydrate	4.155g in 50mL water (X100)
	13		Bacteriological Peptone	(2g per 100mL) 9g in 450mL water in a glass bottle (X10) and autoclaved.
	14		Yeast Extract	0.18g in 100mL water (X100) autoclave

Table 2

3 Add 40-45 mL ultrapure water to the powders and give them time to go fully into solution. It may be necessary to warm some chemicals.

4 Depending upon what volume of AU you want to prepare, you will need to calculate how much of reagent 4 (Urea) to make. For 1L AU, you need 100 mL of the 10X reagents so you may want to prepare 1.5 times the volumes in order to be able to accurately transfer 100 mL. Urea should be prepared in a glass bottle of sufficient size for the volume being made. After dissolving the powder and making up to the final volume with ultrapure water, warm up the urea, or use pre-warmed water to dissolve. Due to the endothermic nature of urea dissolving, the liquid becomes sufficiently cold such that it also cools down the mixture of reagents after it is added and will cause reagent 9 to precipitate. Once reagent 9 precipitates out of solution, it is very difficult to reverse the reaction so you will not get a true AU solution and you will need to begin mixing again! However, urea also breaks down with heat to form isocyanates which may be detrimental to bacteria so it is recommended to avoid unnecessary heating.

5 The media components, reagents 13 and 14, can be prepared in glass bottles and autoclaved. Note, the final AU will be filter sterilized since not all reagents are sufficiently heat stable to be sterilized by autoclaving. So it is not essential to autoclave the Peptone or YE but if you plan to keep these media components more than the working day, you should autoclave them and prepare them in glass bottles.

6 Top up the 12 plastic tubes to exactly 50 mL each with more ultrapure water.

7 For reagent 9, Potassium oxalate, transfer 5 mL of the now ready 1000X into a new 50 mL tube and top up to 50 mL to produce the 100X stock.

8 Take ten 10mL stripettes (which can be labelled and reused if the packaging is retained).

9 Being transferring each of the reagents into a 1L beaker or 1L measuring cylinder, one at a time in the order that they appear in the list. (It should work in any order but it can help to prevent precipitation if the order is followed.) Use a stripette to transfer 10 mL for the 100X reagents. Add reagent 9, Potassium oxalate DROPWISE to help avoid precipitation. It will also help to prevent any precipitation if you add in 100-200 mL ultrapure water at the start.

10 Transfer 100 mL of the 10X reagents.

11 Use a Bunsen with the media components (reagents 13 and 14) to maintain sterility of the stocks.

12 After addition of all 14 reagents, top up to 1L with ultrapure water and mix well.

13 Aliquot the AU as desired and freeze for storage longer than 2 days, otherwise keep at 4ºC when not in use.

14 AU contains some reagents which cannot be autoclaved, so the final solution must be filter sterilized using a 0.2 um filter before being used in an experiment.

15 Disposal: AU can be flushed down the sink with plenty water. AU that has been used to grow bacteria or harbours phages must be collected into an autoclavable container.