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50mM $CaCl_2$

Manufacturing Site:	Manufacturing Date:	Manufacturing Batch #:

Purpose: 50mM $CaCl_2$ is used for bacterial transformation, prior to the heat shock state.

Note: For this set of instructions, you will make a volume of 50mM $CaCl_2$, aliquoting 10mL into each 15mL conical tube.

Materials before you start:

1. 50mM $CaCl_2$ - this comes in different forms, anhydrous, mono, di-hydrate. Read the label carefully so you know which one you are using.

Materials for making 50mM CaCl2 include:

- ❏ beaker of appropriate size
- ❏ Scale
- ❏ Stir bar
- ❏ Stir plate
- ❏ Graduated cylinder 500 mL, 1000 mL, 2000 mL
- ❏ Disposable pipettes - 10 mL
- ❏ Pipette pump
- ❏ Vacuum pump
- ❏ 0.2µM filter w/bottle
- ❏ 15 mL conical tubes
- ❏ Rack to hold 15 mL conical tubes.
- ❏ Avery labels 5160 (see approved vendor list) or equivalent

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GMP step: Please initial on steps that require verification (steps with additional columns). Two technicians are needed for those steps. One to perform the action and initial and one to verify by checking and initializing.

Procedure:

1. **Determine amount needed:** Use this table to scale your final volume of this reagent. Circle which variety of $CaCl_2$ you are using and use the appropriate amount.

Reagent $CaCl_2$	**Lot #**	**Exp Date**	**Final Concentration**	**Grams of $CaCl_2$ for a Final Volume Of 1L**	**Desired final volume _____ L**
Anhydrous			50 mM	5.55g	
Monohydrate			50 mM	6.45g	
Di-hydrate			50 mM	7.35g	
Distilled water			N/A	≈1L	
			Final Volume	1L	_____L

For 1000 mL procedure:

2. Obtain a 1000 mL beaker.		
3. Place beaker on a stir plate with stir bar in the beaker.		
4. Put 500mL dH_2O into the beaker. Start stirring.	**Technician Initial:**	**Verifier Initial:**
5. Weigh out the calculated amount of $CaCl_2$ and write here: _____g		
6. Slowly add the $CaCl_2$ to the beaker. Stir until the solution is clear.		
7. Transfer solution to a 1000 mL graduated cylinder capable of appropriate size.		

8. QS with dH2O, or add enough water to bring the solution up to **1000 mL**. QS= "quantity sufficient".	**Technician Initial:**	**Verifier Initial:**
9. Transfer solution back to beaker/stir plate and continue stirring for another 2 minutes		
10. Pour the solution into a 0.2 µm filter with bottle. Filter the 50mM $CaCl_2$ using a vacuum pump.		
11. Fill each 15 mL conical tube with 10 mL of $CaCl_2$.	**Technician Initial:**	**Verifier Initial:**
12. Do a visual check that each tube contains about 10 mL of $CaCl_2$.	**Technician Initial:**	**Verifier Initial:**
13. This will make 47-50 tubes.		
10. Label each tube appropriately (see labeling on next page.)		
11. Fill out the table for Inventory below.		
12. Fill out the Work Product found here with your intials and batch number:		

Inventory:

Number of Tubes that were made:	**# tubes:**	**Technician Initial:**	**Verifier Initial:**

QC for 50mM CaCl2.

The quality test for this solution is transformation of bacteria using a plasmid (see www.babec.org for a procedure.) Please ask the BioSCOPE project manager if you are in need of plasmids or bacteria.

Labeling:

Labeling of each conical tube. See below. Avery labels work well here.

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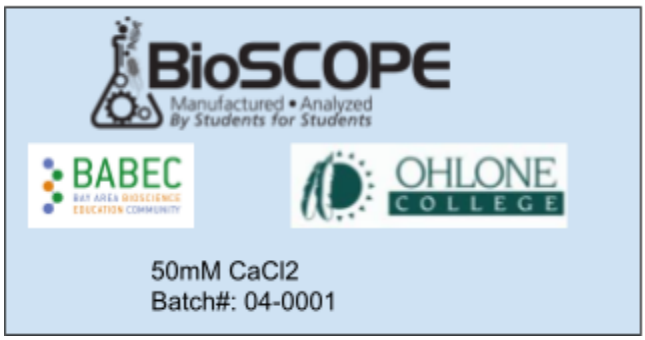
Name & logo of Manufacturer:
Dist by: BABEC logo
BioSCOPE logo
Product Name
Batch #:

Example:



Note that **"04"** is the manufacturing site code for Ohlone College and "**0001**" is the batch number.

BioSCOPE labels are premade. Please ask the project manager for more labels if needed.

I certify that these reagents have been prepared with best efforts to adhere to Manufacturing Site: _____'s Standard Operating Procedures and with all necessary safety precautions.			
	Name:	**Signature:**	**Date:**
Technician:			
Witness:			