iGEM2013 – Microbiology – BMB – SDU

Title: Kinating probe for northern blot **Date issued:** 2013.08.28

SOP number: SOP0028_v01 Review date: 2013.08.28

Version number: 01 Written by: PRA

1. Purpose

To kinate probe with radioactive phosphorus.

2. Area of application

All DNA and RNA probes

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for
			approval/rejection
Heating block		•	
Pippette p10		•	
		•	
		•	
		•	
		•	
		•	

4. Materials and reagents – their shelf life and risk labelling

Name	Components	Supplier / Cat. #	Room (hallway storage)	Safety considerations
RNA or DNA probe		Sigma Aldrich		
T4 polynucleotide		NEB/M0201S		

kinase			
T4 polynucleotide			
kinase buffer			
γ-[³² P]-ATP			
	_		_

5. QC - Quality Control

6. List of other SOPs relevant to this SOP

SOP0025_v01_Growth for RNA purification - IPTG inducible plasmid SOP0026_v01_Growth for RNA purification - ara inducible plasmid SOP0027_v01_RNA purification SOP0028_v01_Nothern blotting SOP0030_v01_Gel for northern blotting

7. Environmental conditions required

8. **Procedure**

- 1. Mix the following (add γ -[³²P]-ATP and enzyme last)
 - 1. 1 μL 10 x polynucleotide kinase buffer
 - 2. $1 \mu L 10 \mu M$ oligo probe
 - 3. $2 \mu L \gamma [^{32}P] ATP$
 - 4. 0.5 μL polynucleotide kinase
 - 5. H₂O to 10 μL
- 2. Incubate at 37°C for 30 min
- 3. Heat inactivate at 80°C for 10 min
- 4. Store at -20°C in box for radioactive compounds

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z)	Remarks

10. Time consumption

- 1 hours
- 15 min hands on time

11. Scheme of development

Date / Initials	Version No.	Description of changes
13.08.28 / PRA	01	The SOP has been written
13.08.28 /	01	The SOP has been approved

12. Appendices