

Protocol	#B.1
Title	BOMB TRI reagent
Keywords	Total RNA extraction, TRI, TRIZOL
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#### Summary

This protocol describes the preparation of the TRI reagent which is used for total RNA extraction. The procedure is a slightly modified version of the recipe published by Chomczyński and Sacchi in 1987 [1].

#### Chemicals

Name	Provider	PN	MW [g/mol]	Safety codes	
8-Hydroxyquinoline (C₃H7NO)	Roth Chemicals	2557.1	145.16	H: 302-412 Warning P: 270-273	
Acetic acid (CH₃COOH)	Roth Chemicals	6755.2	60.05	<b>Danger</b>	H: 314-226-290 P: 210-280- 303+361+353- 305+351+338-310
Ammonium thiocyanate (CN₂H₄S)	Roth Chemicals	4477.4	76.12	(t) Warning	H: 302+312+332-412 P: 220-273-280- 309+311-EUH032
Glycerol (C₃H <sub>8</sub> O₃, 100%)	Roth Chemicals	3783.3	92.09	n.a.	n.a.
Guanidine isothiocyanate (GITC, C₂H₅N₄S)	Roth Chemicals	2628.4	118.16	<b>(</b> Warning	H: 302+H312+H332- 412 P: 273-P280-302+352- 304+340-312-EUH032
Phenol (C₅H₅O)	Roth Chemicals 0040.2 94.11		H: 301+311+331-314- 341-373-411 P: 202-280- 301+330+331- 303+361+353- 305+351+338- 308+313		
or Water-saturated phenol pH 4.5-5	Roth Chemicals	A980.3	mixture	<b>Danger</b>	H: 301+311+331-314- 341-373-411 P: 201-280-304+340- 305+351+338-310
Sodium acetate anhydrous (C <sub>2</sub> H <sub>3</sub> NaO <sub>2</sub> )	Roth Chemicals	6773.2	82.03	n.a.	n.a.

Please consult appropriate MSDS information before working with these chemicals! Use lab coat, gloves and eye protection at all times! The chemicals are available from other providers as well. No preference is given to the indicated vendors.





### **Buffers and solutions**

3 M sodium acetate (~85 ml for 1 l TRI reagent) - can be stored at RT for at least 6 months (adjust pH to 5.0 with acetic acid)

4 M guanidine thiocyanate (200 ml for 1 | TRI reagent) – can be stored at RT for at least 6 months

5 M ammonium thiocyanate (80 ml for 1 | TRI reagent) - can be stored at RT for at least 6 months

Water-saturated phenol pH 4.5-5 - can be stored at 4 °C for at least 6 months

Water-saturated phenol can be also prepared from crystalline phenol with the protocol below

Step	Phenol saturation with water	Time	$\checkmark$
1	Dissolve 1 kg of the crystalline phenol in a water bath at 50 °C		
$\triangle$	Phenol is highly toxic, perform all manipulations with gloves and lab coat in a fume hood!	~4 h	
2	Transfer the dissolved phenol to the preheated 2 l bottle		
3	Add $\sim$ 100 ml portions of warm water to the phenol and shake vigorously		
4	Stop adding water until maximum saturation is observed		
$\underline{\wedge}$	Phenol solution turns milky after shaking when saturated		
5	Distribute the saturated phenol in two 1 litre bottles and store at 4 $^{\circ}$ C	O/N	
6	Next day after the phases separate discard the upper water phase		
7	Carefully estimate the phenol volume		
8	Add 8-Hydroxyquinoline up to 0.1% (mass to volume)		
$\wedge$	8-Hydroxyquinoline preserves phenol from oxidation and provides a yellow colour for easier handling		
End			
	Reagent can be stored @ 4 °C for up to few months without any loss in performance		

performance



### **BOMB Preparation of TRI reagent**

Step	TRI reagent				Time	$\checkmark$
1	Mix the separate components in an appropriate size bottle			10 min		
	Solution	2.5	11	0.5 l	0.1	
	Water saturated phenol (supplemented with 8- Hydroxyquinoline)	950 ml	380 ml	190 ml	38 ml	
2	4 M Guanidine thiocyanate	500 ml	200 ml	100 ml	20 ml	
Z	5 M Ammonium thiocyanate	200 ml	80 ml	40 ml	8 ml	
	3 M Sodium acetate	83.5 ml	33.4 ml	16.7 ml	3.34 ml	
	Glycerol (100 %)	125 ml	50 ml	25 ml	5 ml	
	ddH₂O	up to 2500 ml	up to 1000 ml	up to 500 ml	up to 100 ml	
$\triangle$	Phenol is highly toxic, perform all mo	nipulations w	ith gloves and	d lab coat in d	a fume hood!	
3	Mix on a magnetic stirrer for 15-20 r	nin			20 min	

End	Distribute into dark bottles	<b>~35 m</b> (15 min har	i <b>n</b> nds-on)
4	Check the pH of the prepared TRI reagent – should be acidic pH ~5	5 min	
5	Wix of a magnetic stiffer for 15 20 mill	20 11111	

Store @ 4 °C

# Troubleshooting

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Problem	Solution
TRI spillage	Have a bottle of PEG 300 or 400 nearby and use this to wipe off immediately
	and repeatedly

# **Exemplary results**



Fig 1: Prepared TRI reagent before and after addition of chloroform.

## References

1. Chomczynski P. Single-step method of RNA isolation by acid guanidinium extraction. 1987;159: 156– 159. doi:10.1016/0003-2697(87)90021-2

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