How will you prepare the N-acetylcysteine (NAC) concentration?
Dear all,

I would like to use N-acetylcysteine (NAC) to rescue the ROS-inudced cell death caused by a drug treatment. However, I found the final concentration of NAC people used widely ranged from 1mM to 25 **mM.**

I dissolve the NAC in DMSO and from the solubility provided by Selleck (32mg in 1ml DMSO), we can only get the maximal stock concentration at 196.09 mM. Considered the toxicity of DMSO, I usually use DMSO at 0.1% (1:1000 dilution)

Here comes the problem, if I do so, this means I could only have 196.09uM in my culture system.

How could I adjust final concentration to 10mM (dissolved in DMSO) like the concentration used in papers?

Or any other ways to get the 10mM final concentration?

Thank you so much,

Wei

DMSO | Acetylcysteine | Solubility

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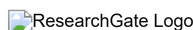


Most recent answer

Wei Zhang
The University of Manchester

8th Aug, 2022

I recently prepared a another batch of NAC. It worked well as well.

(1) Dissolve the NAC power in D-PBS (cell culture level) to a stock concentration of 150mM

(2) Adjust the pH to 7.2-7.4

(3) Filter it through 0.22um sterile syringe filter

(4) Aliquote 1ml in 1.5ml EP tube and stored in -20 freezer (3 weeks at least are absolutely fine)

Hope it helps.

Cite

All Answers (5)

Qi Lei

10th Sep, 2021

10th Sep, 2021

Peking University

"In all experiments, NAC (final concentration: 20 mM, unless otherwise specified) was dissolved in H2O, and pH was adjusted to 7.4 by adding NaOH. Aliquots were stored at –20°C for 1 month maximum." i think H2O is better than DMSO. but given the maximal concentration, H2O is still unmet.

Pilipow, K. *et al.* Antioxidant metabolism regulates CD8+ T memory stem cell formation and antitumor immunity. *JCI Insight* **3**, e122299.

Cite 1 Recommendation

Mohamed Khedawy
National Institute of Oceanography and Fisheries

14th Sep, 2021

NAC is water soluble, so it is easy to use in any solution. I would be careful with standard buffers, since many proteins and vitamins that are added to the buffers make ROS themselves (sugar, for example). Depending on the cells you are using, consider that they may not take NAC up at all, it may work only on the outside of the cell.

Since NAC is acidic, be sure to buffer your solution to a neutral pH with NaOH, to not add an additional stress.

I used an end-concentration of 2.5 mM, but I had packed RBC.

Cite

Mohamed Khedawy
National Institute of Oceanography and Fisheries

14th Sep, 2021

You can dissolve NAC either in Water or in DMSO upto 100mM (stock). I use 100mM stock in DMSO. Optimum concentration of NAC would vary from cell line to cell line. Usually 500uM to 1mM of NAC in the cell culture works with most of the cell lines.

Cite 1 Recommendation

Wei Zhang
The University of Manchester

1st Jun, 2022

Thanks for all the answers. They are all helpful.

I would like to update this question for reference.

I dissolved the NAC powder in D-PBS to prepare a stock of 100 mM, which was then passed through a 0.22um sterile syringe filter and stored in -20 for 1 week.

For OE19 cells, I used 1mM as the final concentration and changed the culture medium every day. No tocxicity observed in terms of cell density compared to the control.

Thanks again for all.

Cite

Similar questions and discussions

Does anyone know how to make stock solution of N acetyl sistein (NAC) to use in culture cells?

Question 6 answers

Asked 14th Nov, 2017

Agustina Setiawati

NAC is an antioxidant molecule that inhibit ROS activity by scavenging activity. Considering cell toxicity and morphological changes, what solvent I should use in culture cells (PBS, UPW or any other) and how much does maximal concentration I should apply?

View

Can anyone give me advice on using N-Acetyl Cysteine in Cell Culture?

Question 10 answers

Asked 2nd Feb, 2016

Eric Franklin

Hi everyone, I have been recently looking at the effect of hypoxia on proliferative capability of different cell types and given that the primary cells I have tested preferred lower oxygen concentrations I've tried using NAC treatments to determine if this preference is due to lower ROS in the low O2 incubator. I have used 5mM concentration over a 7 day period (changing media every 2-3 days) but have gotten sporadic results. Does anyone have advice for how I can alter this to better suit my aims as I have been hard pressed to find anyone using NAC for longer than 48 hrs? Thanks in advance.

View

View

What's good positive control for ROS analysis?

Question 14 answers

Asked 28th Jan, 2020

Sandeep Kumar

I'm using h2o2 as a positive control for h2dcfda assay for the detection of ROS. There's five fold less (20%) Ros than untreated control (100%) Thp1 cells. I'm treating cells for 24 Hours. Can I use some other controls which are less toxic and less expensive.

View

50 mM prepartion of N-Acetyl-L-cysteine?

Question 4 answers

Asked 3rd Jun, 2018

Hayaa Alhuthali

Hello all

I have an agent that increase the level of reactive oxygen species, I would like to do co-treatment with free radical scavengers (N-Acetyl-L-cysteine) to see if this will rescue cells from death. MY cells is at 5 x 10^5 cells/ml.

For my agent I prepared working solution (20.4 µM) and then I to culture my cells in final concentration 0.4 µM, I add 20 µl of this working solution to my cells (1 ml). Now I need to do co- treatment with 50mM or 10mM of N-Acetyl-L-cysteine…my questions is how can I prepare that and how much do I need to add to my cells to get these concentration

View

How to calculate the log2 fold change?

Question 24 answers

Asked 7th Nov, 2017

Ganesh Ambigapathy

I have 3 groups. 1. Control 2. Disease 3. Treatment. I want to lookup the gene expression btw these groups, compared with control (whether is upregulated or downregulated).

I did real-time qPCR and have ct values. I calculated ∆Ct = Ct[Target]-Ct[Housekeeping] ... and ∆∆Ct = (∆Exp.)-(∆Control) and got the -∆∆Ct log-fold-change. It looks all the values are almost same and not much different between the groups.

My questions are,

1. What I did was right?

2.If I plot a graph what should I mention in y-axis?

3. Is there any other better way to calculate the gene expression results better?

4.How to calculate log2 fold change and does it helps to see the results more clearer?

p.s I have attached the .xls file for your reference.

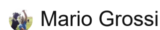
Thanks in advance...!!!

View

Would it be possible to store 4%PFA fixed cells in PBS at 4degrees for many days?

Question 9 answers

Asked 12th May, 2017

 Mario Grossi

Dear RGfriends, I would like to stain primary cells against Ki67-antibody (and more antibodies) at different cell passage (from P=1 to P=6). In order to run the ICC for all samples at the same time, I thought to fix the cells with 4%PFA (10min) and then store them in PBS at 4 degrees until I will collect all samples. Would it be ok? Thank you in advance for your suggestions and help. Looking forward to hearing from you in the near future and in the meantime I wish you a great weekend. Best Regards,

Mario

View

How to reconstitute SiRNA?

Question 4 answers

Asked 20th Sep, 2016

Manisha Dagar

I want to reconstitute my SiRNA. Can i reconstitute it in DEPC treated water or 1X SiRNA buffer is compulsory. If yes, I also want to know the exact composition of this buffer. Can anyone help me out with this.

View

What is the role of NaF in RIPA buffer?

Question 8 answers

Asked 13th Mar, 2015

Zhengrong Zhou

I am looking for the protocol of RIPA buffer in the internet and found many different kinds of RIPA buffer, some have NaF and some without, does anyone know the function of NaF in the RIPA buffer?

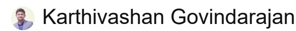
Thank you!

View

How to prepare 40 mM hydrogen peroxide from 30% H2O2 solution ?

Question 65 answers

Asked 24th Aug, 2012



Karthivashan Govindarajan

For H202 radical scavenging assay. How to prepare 40 mM hydrogen peroxide from 30% H2O2 solution ? Thanks in advance.

View