

Adenovirus Protocol

A-195/PBS/5% Sucrose Dialysis and Storage Buffer

A195 Dialysis Buffer

3 Components:

1. 10X A195 Stock Buffer (modified in 2015 the amount per liter to have final concentrations as in the original reference paper. ie "10X" Stock is not truly a 10X stock to get to the published final concentrations so we need to add a little more than 1/10.) This concentrate contains EDTA, MgCl₂, Tris, EtOH, and PS-80 and is not the final dialysis buffer.)
2. 50g per Liter Sucrose
3. 1 packet per Liter of Sigma-P3813 PBS pH 7.4

| | <u>Per 4L</u> | <u>3L</u> | <u>2L</u> | <u>Per 1L</u> |
|---------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| 10X A195 Stock Buffer | 432ml | 324ml | 216ml | 108ml |
| Sucrose | 200g | 150g | 100g | 50g |
| Sigma-P3813 PBS pH7.4 | 4 packets | 3 pk | 2pk | 1 pk |
| Pyrogen Free ddH ₂ O | ~3568ml (to 4L final) | ~2676ml (to 3L final) | ~1784ml (to 2L final) | ~892 ml (to 1L final) |

A-195 10X Conc. stock solution - 4 Liters (~ 10X)

62.08g Histidine

315.2g Tris HCl

Then Add....

40ml 1M MgCl₂

40ml 20% PS-80

40ml 100mM EDTA

200ml 100% Ethanol (200 proof)

pH to 7.4 with 10N NaOH. QS to 4L with endotoxin free ddH₂O

Filter sterilize it using a 0.2um filter.

Stock Solutions:

1M MgCl₂

203.3g MgCl₂

QS to 1L with endotoxin free ddH₂O

Filter sterilize it using a 0.2um filter.

20% PS-80

200ml PS-80 (also called Tween)
800ml endotoxin free ddH₂O

Using a glass beaker, heat solution with low heat using a stir/heating plate until it gets into solution.

Filter sterilize it using a 0.2um filter.

100mM EDTA

200ml 0.5M EDTA
800ml endotoxin free ddH₂O

Filter sterilize it using a 0.2um filter.

Final Concentration in A195 Dialysis Buffer:

10mM Tris
75mM NaCl
5% Sucrose (w/v)
0.020% PS-80 (w/v)
1mM MgCl₂
100uM EDTA
0.5% EtOH (v/v)
10mM His
(pH at 25°C = 7.4)

Reference:

A195 Buffer: [Evans RK](#), [Nawrocki DK](#), [Isopi LA](#), [Williams DM](#), [Casimiro DR](#), [Chin S](#), [Chen M](#), [Zhu DM](#), [Shiver JW](#), [Volkin DB](#). *Development of stable liquid formulations for adenovirus-based vaccines.* [J Pharm Sci.](#) 2004 Oct;93(10):2458-7