Ready-to-use incubation buffer solutions based on Aurion BSA-c™

PRODUCT INFORMATION

AURION BSA-c™ is an acetylated and (partly) linearized form of Bovine Serum Albumin, having an increased net negative charge by acetylation of amino groups of basic amino acids. AURION BSA-c™ will improve signal-to-noise ratios independent of the immunodetection system being used.

TWEEN-20® is a non-ionic surfactant, a detergent with hydrophobic alkyl side chains. When other hydrophobic components (proteins, antibodies, gold conjugates etc.) are present these components are likely to get embedded in micellar structures. One of the main effects is the suppression of hydrophobic (background) interactions. It acts as a surfactant and thus will enhance reagent spreading in (automated) immuno incubation procedures

AURION ready-to-use incubation buffer solutions are available in two compositions:

900.099-PBS
PBS (10mM Phosphate buffer, 150mM NaCl) +
0.1% BSA-c™ + 15mM NaN_3, pH 7.4
It is recommended to include a protein block step in the immuno incubation procedure (See protocol nr 1)

900.099-PBST
PBS (10mM Phosphate buffer, 150mM NaCl) +
0.1% BSA-c™ + 0.1% TWEEN-20® + 15mM NaN_3, pH 7.4
A protein block step may be omitted from the immuno incubation procedure (See protocol nr 2 and the Important Note)

AURION ready-to-use buffer solutions are supplied in 1000 ml quantities. Store at 4°C.

GENERAL REMARKS

It should be noted that attempts to reduce background may have an influence on the specific reactions too, since they obey the same laws of organic chemistry.

A special AURION NEWSLETTER dealing with the topic of background is available on https://aurion.nl

Important Note:

TWEEN-20® is a detergent. Surface forces may affect/remove existing hydrophobic complexes in/from the specimen and encapsulate them in micelles. When this is the antigen, this will have a negative effect on signal intensity.
In biological specimens TWEEN-20® may have a negative effect on (ultra)structure.

SUGGESTED INCUBATION PROTOCOL nr 1:

Ready-to-use incubation buffer 900.099-PBS:
PBS (10mM Phosphate buffer, 150mM NaCl) +
0.1% BSA-c™ + 15mM NaN_3, pH 7.4

1. Aldehyde inactivation
   For on section labelling: PBS with 50 mM Glycine, pH 7.4, 20 minutes
   For pre-embedding: 0.1M PB with 0.1% NaBH4, wash thoroughly in PB to remove excessive NaBH4

2. Block step
   PBS with 5% BSA, 5% normal serum (same species as secondary antibody source) and 0.1% cold water fish skin gelatin for 10-30 minutes. This block buffer is also available as ready-to-use solution from Aurion (see auxiliary products).

3. Wash step
   Incubation buffer 3x5 minutes

4. Primary antibody incubation
   1-5 µg/ml primary antibody in incubation buffer, 30-60 minutes

5. Wash step
   Incubation buffer 3x10 minutes / 6x5 minutes(EM)

6. Secondary antibody incubation
   ImmunoGold/ fluorescent/ enzyme conjugated reagent diluted in incubation buffer, 30 minutes - 2hrs.

   Note: For Streptavidin reagents in a 3-step system:
   Incubate in biotinylated secondary antibody according to step 4, rinse according to step 5 and proceed with step 6

7. Wash step
   Incubation buffer 3x10 minutes / 6x5 minutes (EM)

8. Wash step
   PBS 3x5 minutes

9. Optional for ImmunoGold (Silver) Staining: post fixation
   2% Glutaraldehyde in PBS, 10 minutes
   Wash thoroughly in PBS

10. For IGSS proceed with silver enhancement using:
    AURION R-Gent SE-LM for light microscopy and bio-assays or
    AURION R-Gent SE-EM for EM applications
    See package insert for instructions

SUGGESTED INCUBATION PROTOCOL nr 2:

Ready-to-use incubation buffer 900.099-PBST:
PBS (10mM Phosphate buffer, 150mM NaCl) +
0.1% BSA-c™ + 0.1% TWEEN-20® + 15mM NaN_3, pH 7.4

1. Optional: Aldehyde inactivation
   When aldehydes (e.g., formalin) are being used for chemical fixation of the specimen, free aldehyde groups are blocked in PBS with 50 mM Glycine, pH 7.4, 20 minutes
   Note: For pre-embedding the use of 0.1M PB with 0.1% NaBH4 is recommended, wash thoroughly in PB to remove excessive NaBH4

2. Optional: Protein block step
   Note: The proteins used in this block step may likely be (in part) removed by the subsequent washing steps in buffer with TWEEN-20®. A protein block step is therefore for many applications not necessary. Leaving out this blocking step will significantly reduce the length of the immuno incubation procedure.

   Recommended blocking buffer composition: PBS with 5% BSA, 5% normal serum (same species as secondary antibody source) and 0.1% cold water fish skin gelatin for 10-30 minutes. This block buffer is also available as ready-to-use solution from Aurion (see auxiliary products).

3. Wash step
   Incubation buffer 3x5 minutes

4. Primary antibody incubation
   1-5 µg/ml primary antibody in incubation buffer,
   30-60 minutes

5. Wash step
   Incubation buffer 6x5 minutes

6. Secondary antibody incubation
   ImmunoGold/ fluorescent/ enzyme conjugated reagent diluted in incubation buffer, 30 minutes - 2hrs.

   Note: For Streptavidin reagents in a 3-step system:
   Incubate in biotinylated secondary antibody according to step 4, rinse according to step 5 and proceed with step 6

7. Wash step
   Incubation buffer 6x5 minutes

8. Wash step
   PBS 3x5 minutes

9. Optional for IGSS: post fixation
   2% Glutaraldehyde in PBS, 10 minutes

10. For IGSS proceed with silver enhancement using:
    AURION R-Gent SE-LM for light microscopy and bio-assays

AUXILIARY PRODUCTS

CODE	DESCRIPTION
900.099	AURION BSA-c™ (10%), 30ml
900.022	AURION BSA-c™ (10%), 100ml
905.001	Basic blocking solution, 30ml
905.002	Blocking solution for Goat gold conj., 30ml
905.003	Blocking solution for Rabbit gold conj., 30ml
905.004	Blocking solution for Sheep gold conj., 30ml
905.005	Blocking solution for Donkey gold conj., 30ml
900.011	Bovine serum albumin fraction V, 25g
900.033	Cold Water Fish Skin Gelatin (40%), 10ml
900.066	Normal Rabbit Serum, 5ml
900.077	Normal Goat Serum, 5ml
900.111	Normal Sheep Serum, 5ml
900.122	Normal Donkey serum, 5ml
500.011	AURION R-GENT SE-LM, 60ml
500.022	AURION R-GENT SE-LM, 250ml
500.033	AURION R-GENT SE-EM, 30ml
500.044	AURION R-GENT SE-EM, 90ml

AURION
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