immusmol

Instructions for use

STAIN*perfect*

Immunostaining kit A

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REF SP-A-1000 Σ 40 +2 +8 °C RUO

1. Intended use and principle of the test

STAIN*perfect* immunostaining kits provide researchers with optimal conditions to detect and visualize small molecules using compatible ImmuSmol antibodies. Unlike proteins, small molecules require specific sample preparation and staining processes, so as to prevent their degradation and preserve the initial specificity and affinity of the antibodies.

The STAIN*perfect* immunostaining kit A is to be used in combination with specific ImmuSmol antibodies, as mentioned on antibody product pages. The kit contains all necessary reagents for the wash, fixation, permeabilization, stabilization and saturation of samples. Flexible and easy-to-use, the kit comes with validated step-by-step protocols for the staining of cell cultures, whole mounts and tissue sections. Furthermore, the kit is compatible with all standard secondary antibodies.

2. Advice on handling the test

2.1 Complaints

In case of complaints, please address to the manufacturer a written report containing all data as to how the test was conducted, the image obtained and a copy of the laboratory workbook. Please contact the manufacturer to get a reclamation form.

2.2 Warranty

This staining kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. The kit is intended for profesional use only. The principles of Good Laboratory Practice (GLP) must be followed. Any alteration of the kit or the staining procedure, as well as the usage of reagents from different lots may have a negative influence on staining quality, and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

2.3 Disposal

Residual substances and/or all remaining chemicals, reagents and ready-to-use solutions are special refuse. Inform the responsible authorities or refuse disposal enterprises about the removal of special refuse. Disposal is subject to fereral and national laws and regulations. Legal basis for the disposal of special refuse is the economic cycle and waste law. The appropriate safety datasheet is available for download on our website, on the product page. The safety datasheet correspond to the standard: ISO 11014-1.

2.4 Interference

Do not mix reagents and solutions from different lots. Consider storage conditions. Inappropriate handling of samples or deviations from the test regulation can affect the results. Do not use kit components beyond the expiration date. Avoid microbiological contamination of the reagents. Consider incubation periods and wash references.

2.5 Precautions

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hands thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. All reagents should be treated as potential biohazards in use and for disposal.

3. Storage and stability

- Store the reagents at 2 - 8 °C until expiration date.
- Do not use components beyond the expiry date indicated on the kit labels.
- Do not mix components from various lots within an individual assay.

4. Materials

4.1 Content of the kit

Ref.	Symbol	Color	Reagent	Format	Status
SP-A-1001	WASH 1 25X	Light purple	Wash Solution 1$^{\Delta}$ (25X)	50 mL	Dilute in distilled water
SP-A-1002	WASH 2 25X	Green	Wash Solution 2$^{\Delta}$ (25X)	50 mL	Dilute in distilled water
SP-A-1003	WASH 3 25X	Brown	Wash Solution 3$^{\Delta}$ (25X)	20 mL	Dilute in distilled water
SP-A-1004	FIX-BUFF 25X	Blue	Fixation Buffer (25X)	20 mL	Dilute in distilled water
SP-A-1005	FIX-REAG	Blue	Fixation Reagent*	2x5 mL	Dilute in Fixation buffer (1X)
SP-A-1006	PERM-SOLN	Red	Permeabilization Solution	22 mL	Depending on the sample
SP-A-1007	STAB-BUFF 25X	Yellow	Stabilization Buffer (25X)	20 mL	Dilute in distilled water
SP-A-1008	STAB-REAG	Yellow	Stabilization Reagent	6 vials (powder)	Reconstitute in Stabilization buffer
SP-A-1009	SAT-SOLN	Light grey	Saturation Solution	22 mL	Ready to use
SP-A-1010	AB-DILUT	Black	Ab Diluent	22 mL	Ready to use

$^{\Delta}$ Wash Solutions must be warmed up at 37°C for 30 minutes and skaked before reconstitution **(!)**
* The Fixation Reagent and subsequent Fixation Solution must be handled with care under chemical hood and wastes must be collected as toxic agents.

4.2 Additional materials, which may be required but not provided in the kit

Reagents:
- Water (deionized, distilled, ultra-pure)
- NaCL
- Heparin
- Sucrose
- O.C.T (embedding)
- Mounting medium
- Primary antibody purchased from ImmuSmol and suitable secondary antibody from third-party vendor.

Equipment:
- Vortex mixer
- Shaker (shaking amplitude 3 mm, approx. 600 rpm)
- Calibrated precision pipettes

5. Sample collection

Collect and prepare samples using standard procedures. To prevent any degradation, process samples immediatly after their retrieval.

6. Preparation of immunostaining kit solutions

- Always prepare reagents freshly. Failure to do so may lead to suboptimal results.
- The required total buffer volumes should be calculated beforehand; volumes will depend on the nature of the samples and number of tests to be performed.
- Always wear proper protective clothing and gloves when handling the different solutions.
- Before dilution make sure that buffer does not contain any precipitates.

6.1 Wash Solutions

To achieve the appropriate working concentration, the different wash solutions (i.e. Wash Solution 1, Wash Solution 2 and Wash Solution 3) must be diluted 1/25 with deionized or distilled water (i.e. use 1 mL of wash solution plus 24 mL of distilled or deionized water). Once reconstituted, solutiuons are stable for 1 month at 2-8°C.

6.2 Fixation Solution

As a first step, the Fixation Buffer must be diluted 1/25 in distilled or deionized water (i.e. use 1 mL of Fixation Buffer plus 24 mL of distielled water). Then the Fixation Reagent must be diluted in Fixation Buffer according to the nature of the sample to be analyzed.

- For perfusion fixation and whole mounts samples, use Fixation Reagent diluted 1/10 with Fixation Buffer 1X (i.e. use 1 mL of Fixation Reagent plus 9 mL of Fixation Buffer 1X).
- For cell culture, use Fixation Reagent diluted 1/200 with Fixation Buffer 1X (i.e. use 50 µL of Fixation Reagent plus 9.95 mL of Fixation Buffer 1X).

Caution: The Fixation Reagent contains aldehydes. This aldehyde is toxic ; contact with eyes, skin and mucous membranes should be avoided.

Note: Once prepared, this Solution is not stable and can not be re-used. Failure to do so may lead to suboptimal results.

6.3 Permeabilization Solution

To achieve the appropriate working concentration for safe and efficient permeabilization, the Permabilization Solution must be diluted with Wash Solution 1 (1X) according to the nature of the sample to be analyzed.

- For cell culture, use Permeabilization Solution diluted 1/10 with Wash Solution 1 (i.e. use 1 mL of Permeabilization Solution plus 9 mL of Wash Solution 1).
- For tissue sections, use Permeabilization Solution diluted 1/2 with Wash Solution 1 (i.e. use 1 mL of Permeabilization Solution plus 1 mL of Wash Solution 1).
- For whole mounts samples, use Permeabilization Solution as a ready-to-use solution .

6.4 Stabilization Solution

As the first step, to achieve the appropriate working concentration for safe and efficient stabilization, the Stabilization Buffer must be diluted 1/25 in distilled or deinozed water and 1 vial of Stablization Reagent must be reconstitute - **just before use** - with 4 mL of Stabilization Buffer. The Stabilization Solution is then ready to use.

Note: Stabilization Solution must be prepared **just before use**. Once prepared, this Solution is not stable and can not be re-used. Failure to do may lead to suboptimal results.

6.5 Stabilization Solution

We recommend to use Immu>Smol primary antibodies at a 1/500 dilution in Antibody Diluent. However, in order to ensure optimal esults, it is highly recommended for the end-user to evaluate the appropriate dilution according to the nature of the samples (dilution range 1/250 - 1/1000). Also, other primary antibodies and secondary antibodies (from third parties) can be pepared in the Antibody Diluent provided with the kit.

6.6 Summary

<table>
<tr><th>SAMPLES
SOLUTIONS</th><th>CELL CULTURES</th><th>TISSUE SECTIONS</th><th>WHOLE MOUNTS</th></tr>
<tr><td>Wash Solution 1, 2, 3
Fixation, Stabilization Buffer</td><td colspan="3">1 volume (25X) + 24 volumes distilled or deionized water</td></tr>
<tr><td>Fixation Solution</td><td>1 volume Fixation Reagent + 199 volumes Fixation Buffer (1X)</td><td colspan="2">1 volume Fixation Reagent+ 9 volumes Fixation Buffer (1X)</td></tr>
<tr><td>Permeabilization Solution</td><td>1 volume Permeabilization Solution + 9 volumes Wash Solution 1 (1X)</td><td>Ready-to-use</td><td>1 volume Permeabilization Solution + 1 volumes Wash Solution 1 (1X)</td></tr>
<tr><td>Stabilization Solution</td><td colspan="3">Ready to use after reconstitution</td></tr>
</table>

7. Protocol for cell culture

Proceed to cell plating according to standard protocol. The volumes indicated in the protocol were calibrated for 24-well plates. Adjust volume according to the size of the plate. For information, see table below.

1. Remove cell culture medium and wash the cells with 1mL of Wash Solution 1.

2. Immediately remove Wash Solution 1 and incubate with Fixation Solution for 10 minutes at 20-25°C.

3. Remove Fixation Solution and wash the cells 3 times (3 minutes each) with 1mL of Wash Solution 1.

4. Incubate with 0.5mL of Permeabilization Solution during 10 minutes at 20-25°C.

5. Remove Permeabilization Solution and wash 3 times for 3 minutes each with 1mL of Wash Solution 1.

6. Incubate cells with 0.5mL of Stabilization Solution for 10 minutes at 20-25°C.

7. Wash the cells 3 times for 3 minutes with 1mL of Wash Solution 1.

8. Incubate with 0.5mL of Saturation Solution for 10 min at 20-25°C.

9. After removing Saturation Solution, incubate with 0.5mL of Primary Antibody diluted with Antibody Diluent (recommended dilution **1/500**) overnight at 2-8°C.

10. Weed out the Antibody Solution and wash the cells 3 times for 10 minutes in 1mL of Wash Solution 2.

11. Proceed to Secondary Antibody staining (use instructions provided with the antibody) for 1 hour at room temperature 20-25°C. In the case of immunofluorescence (IF) staining, protect cells from light.

12. Wash cells 3 times for 3 minutes with 1mL of Wash Solution 2, in the dark. Repeat the washes three times with 1mL of Wash Solution 3.

13. Mount cover-slips on glass slides and protect from light (IF). Adapt storage conditons to the mounting medium.

PLATE	VOLUME PER WELL	
	Fixation/Permeabilization/Stabilization/Saturation/Antibody	Wash1, 2, 3
96 wells	0.1mL	0.20mL
48 wells	0.2mL	0.5mL
24 wells	0.5mL	1mL
12 wells	1mL	2mL
6 wells	3mL	4mL

8. Protocol for Tissue sections

The volumes indicated in the protocol were calibrated for 20 to 25g mice. Adjust volume according to the size of the animal and organs.

Tissue sampling & preparation

1. Perfuse the animal according to standard procedure (eg. 10 mL of NaCL 9g/L plus Heparin 10U/ml) and immediately proceed to perfusion fixation with 20mL of Fixation Solution.

2. Remove the organs of interest and place them in 5 mL of Fixation Solution for 3h at 2-8°C.

3. Remove Fixation Solution. Wash 5 times by placing organs in 5mL of Wash Solution 1 for 30 minutes. If needed, complete the wash by placing organs in 5mL of Wash Solution 1 overnight at 2-8°C under slow agitation.

4. Cryoprotect tissues by immersing organs in 10mL of Wash Solution 1 supplemented with 30% (w/v) of sucrose, for 2 days at 2-8°C (change the solution after one day of incubation).

5. Freeze organs according to standard protocol and store samples in -80°C freezer until use.

6. Slice organs. Dry and store the slides in -80°C freezer until their use.

Tissue section staining

7. Dry the slides for 30 minutes at 20-25°C.

8. Wet each slide with 1mL of Wash Solution 1 for 10 minutes at room temperature (20-25°C).

9. Remove Wash Solution 1. Add 0.5mL of Permeabilization Solution (diluted 1/2 (v/v) with Wash Solution 1) on each slide and incubate for 10 minutes at 20-25°C in a wet chamber.

10. Remove Permeabilization Solution and wash slides 3 times for 10 minutes each, with 1mL of Wash Solution 1.

11. Drain the slides. Add 0.5mL of Stabilization Solution on each slide and incubate for 1 hour in a wet chamber.

12. Wash each slide 3 times for 10 minutes with 1mL of Wash Solution 1.

13. Drain the slides. Add 0.5mL/slide of Saturation Solution and incubate for 30 minutes in a wet chamber.

14. Remove Saturation Solution. Add 0.5mL/slide of Primary antibody diluted in Antibody diluent (recommended dilution **1/500**) and incubate overnight at 2-8°C in a wet chamber.

15. Weed out the antibody and wash each slide 3 times for 10 minutes in 1mL of Wash Solution 2.

16. Proceed to Secondary antibody staining (use instructions provided by the manufacturer). In the case of immunofluorescence (IF) staining, protect slides from light.

17. Wash each slide 3 times for 10 minutes with 1mL of Wash Solution 2, in the dark. Repeat the 3 washes with Wash Solution 3.

18. Mount the slides and protect from light (IF). Adapt storage conditons to the mounting medium.

9. Protocol for whole mounts samples

The volumes indicated in the protocol were calibrated for hindbrain plus spinal cord preparation for mouse E14.5 embryo or crayfish whole nervous system. Adjust volumes according to the size of the sample.

1. Collect organs of interest according to standard protocol, using appropriate media.

2. Place sample in 1mL of Fixation Solution for 3h at 2-8°C.

3. Remove Fixation Solution. Wash 5 times by placing tissue in 5mL of Wash Solution 1 for 30 minutes under constant agitation.

4. Put the tissue in 1mL of Permeabilization Solution (ready to use) for 1 hour at 20-25°C under constant slow agitation.

5. Remove Permeabilization Solution. Wash tissue 2 times for 3 minutes with 5ml of Wash Solution 1.

6. Put 1mL of Stabilization Solution for 1 hour at 20-25°C.

7. Wash the tissue 3 times for 3 minutes in 5mL each of Wash Solution 1.

8. Incubate tissue in 1mL of Saturation Solution for 1 hour at 20-25°C under slow constant agitation.

9. After removing Saturation Solution, incubate with 1mL of Primary antibody diluted with Antibody diluent (recommended dilution **1/500**) at least two nights (72 hours) at 2-8°C under slow agitation.

10. Weed out the antibody solution and wash the tissue 5 times for 30 minutes in 5mL of Wash Solution 2 under constant agitation.

11. Proceed to Secondary antibody staining (use instructions provided by the manufacturer) and incubate with 1mL overnight at 2-8°C under slow agitation. In the case of immunofluorescence staining (IF), protect samples from light.

12. Wash samples 3 times for 30 minutes in 5mL of Wash Solution 2 in the dark, with agitation. Repeat the washes three time with Wash Solution 3.

13. Mount the slides and protect from light (IF). Adapt storage conditons to the mounting medium.

PROTOCOL-AT-A-GLANCE

CELL CULTURE (24-well plate)

WASH 1	Remove cell culture medium and wash	1 mL	x 1	1min	20-25°C
1. FIXATION	2.5 µL FIX-REAG (200X) + 497.5 µL FIX-BUFF. 1X	0.5 mL	x 1	10min	20-25°C
WASH 1	40 µL Wash 1 (25X) +960 µL dH2O	1 mL	x 3	3min	20-25°C
2. PERMEABILIZATION	50µL PERM-SOLN (10X) + 450µL Wash 1	0.5 mL	x 1 (Slow agitation)	10min	20-25°C
WASH 1	40 µL Wash 1 (25X) +960 µL dH2O	1 mL	x 3 (agitation)	3min	20-25°C
3. STABILIZATION	STAB-REAG + STAB-BUFF	0.5 mL	x 1	10min	20-25°C
WASH 1	40 µL Wash 1 (25X) +960 µL dH2O	1 mL	x 3 (agitation)	3min	20-25°C
4. SATURATION	Ready to use	0.5 mL	x 1 (Slow agitation)	30min	20-25°C
5. PRIMARY ANTIBODY	2 µL Primary antibody + 998 µL AB-DILUT	0.5 mL	x 1 (Slow agitation)	O / N	2-8°C
WASH 2	40 µL Wash 2 (25X) + 960 µL dH2O	1 mL	x 3 (agitation)	10min	20-25°C
6. SECONDARY ANTIBODY	Secondary antibody not provided	0.5 mL	x1 (Protect from light)	1hour	20-25°C
WASH 2	40 µL Wash 2 (25X) + 960 µL dH2O	1 mL	x 3 (Agitation - Protect from light)	3 min	20-25°C
WASH 3	40 µL Wash 3 (25X) + 960 µL dH2O	1 mL	x 3 (Agitation - Protect from light)	3min	20-25°C
7. MOUNTING & IMAGING	Mounting media not provided				

PROTOCOL-AT-A-GLANCE

TISSUE SAMPLING & PREPARATION (MOUSE BRAIN)

1. PERFUSION FIXATION	NaCL 9g/L + Heparin 10U/ml (not provided)	10mL	x 1	intracardiac perfusion according to standard protocol	
	2 mL FIX-REAG (10X) + 18 mL FIX-BUFF 1X	20mL	x 1	intracardiac perfusion according to standard protocol	
2. POST-FIXATION	0,5 mL FIX-REAG (10X) + 4,5 mL FIX-BUFF 1X	5 mL	x 1	3h	2-8°C
WASH 1	200 µL Wash 1 (25X) + 4,8 mL dH2O	5mL	x 5	30min	20-25°C
CRYOPROTECTION	Wash 1 + 30% (w/v) sucrose	10 ml	x 2 (Slow agitation)	24h	2-8°C
FREEZING					-80°C
3. SLICING & STORAGE	Slice organs, dry and store the slides in -80°C freezer until use.				-80°C

PROTOCOL-AT-A-GLANCE

TISSUE SECTION STAINING

	1. DRYING SLIDES				30min	20-25°C
	WASH 1	40 µL Wash 1 (25X) + 960 µL dH2O /slide	1mL	x 1	10min	20-25°C
	2. PERMEABILIZATION	PERM-SOLN diluted 1/2 (v/v) with Wash 1	0.5 mL	x 1 (in wet chamber)	10min	20-25°C
	WASH 1	40 µL Wash 1 (25X) + 960 µL dH2O	1mL	x 3	10min	20-25°C
	3. STABILIZATION	STAB-REAG + STAB-BUFF	0.5 mL	x 1	1hour	20-25°C
	WASH 1	40 µL Wash 1 (25X) + 960 µL dH2O	1mL	x 3	10min	20-25°C
	4. SATURATION	Ready to use	0.5 mL	x 1 (in wet chamber)	30min	20-25°C
	5. PRIMARY ANTIBODY	1 µL Primary antibody + 499 µL AB-DILUT	0.5 mL	x 1 (in wet chamber)	O/N	2-8°C
	WASH 2	40 µL Wash 2 (25X) + 960 µL dH2O	1mL	x 3	10min	20-25°C
	6. SECONDARY ANTIBODY	Secondary antibody not provided	0.5 mL	x 1	1hour	20-25°C
	WASH 2	40 µL Wash 2 (25X) + 960 µL dH2O	1mL	x 3 (Protect from light)	10min	20-25°C
	WASH 3	40 µL Wash 3 (25X) + 960 µL dH2O	1mL	x 3 (Protect from light)	10min	20-25°C
	7. MOUNTING & IMAGING	Mounting media not provided				

PROTOCOL-AT-A-GLANCE

WHOLE MOUNTS

1. FIXATION	100 µL FIX-REAG (10X) + 900 µL FIX-BUFF (1X)	1mL	x 1	3h	2-8°C
WASH 1	200 µL Wash 1 (25X) + 4.8 mL dH2O	5mL	x 5 (Agitation)	30min	2-8°C
2. PERMEABILIZATION	Ready to use	1mL	x 1 (Slow agitation)	1h	20-25°C
WASH 1	200 µL Wash 1 (25X) + 4.8 mL dH2O	5mL	x 2 (Agitation)	3min	20-25°C
3. STABILIZATION	STAB-REAG + STAB-BUFF	1mL	x 1 (Protect from light)	1h	20-25°C
WASH 1	200 µL Wash 1 (25X) + 4.8 mL dH2O	5mL	x 3 (Agitation)	3min	20-25°C
4. SATURATION	Ready to use	1mL	x 1 (Slow agitation)	1h	20-25°C
5. PRIMARY ANTIBODY	2 µL Primary antibody + 998 µL AB-DILUT	1mL	x 1 (Slow agitation)	72h	2-8°C
WASH 2	200 µL Wash 2 (25X) + 4.8 mL dH2O	5mL	x 5 (Agitation)	30min	20-25°C
6. SECONDARY ANTIBODY	Secondary antibody not provided	1 mL	x 1 (Slow agitation)	O/N	2-8°C
WASH 2	200 µL Wash 2 (25X) + 4.8 mL dH2O	5mL	x 3 (Agitation - Protect from light)	30min	20-25°C
WASH 3	200 µL Wash 3 (25X) + 4.8 mL dH2O	5mL	x 3 (Agitation - Protect from light)	30min	20-25°C
7. MOUNTING & IMAGING	Mounting media not provided				

Symbols

	Contains sufficient for <n> tests		Manufacturer		Storage temperature
	Catalogue number		Batch code		Expiry date
	Caution		Content		Consult instruction for use !
	For research use only				

Contact

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