Staining with P-RANbodies

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Abstract

Each P-RANbody consists of a HRP reporter and a nanobody that binds to antigen(s). This is a protocol to stain cells and tissues with P-RANbodies. The method is similar to common immunostaining protocols. However, this protocol focuses on some important tips.

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Guidelines

The method is similar to common immunostaining protocols, but requires some knowledge.

1) Endogenous peroxidase activity can be found in various cells and tissues. It is critical to inactivate this activity before incubating with P-RANbodies.

2) P-RANbodies contain HRP. HRP is sensitive to sodium azide, a common preservative.

3) TSA is a reagent which often requires extensive washing after reaction.

For questions or discussion, please post them on the RANbody forum.

https://www.protocols.io/groups/ranbody

Before start

P-RANbody plasmids can be obtained from Addgene.

https://www.addgene.org/browse/article/28192638/

Materials

TSA Plus reagents (various types) View by Perkin Elmer

ProClin 150 View by Millipore Sigma

Protocol

Step 1.

Fix cultured cells with with 4% (wt/vol) paraformaldehyde/PBS at 4 °C for 15 min.

The method is essentially same as common immunocytochemical staining. This fixation should be optimized if necessary.

Step 2.

Treat cells with 0.1% (w/v) Triton X-100/PBS supplemented with 0.3% (w/v) H_2O_2 at 4 °C for 15 min, Some cells or tissues might show endogenous peroxidase activity. In this case, incubate cells with 0.3% H_2O_2 for a long time (eg, a couple of hours to overnight).

In some cases, it is also possible to perform this step with 0.3%H_2O_2 in methanol at -20C for overnight. However, it depends on the RANbody-antigen reaction.

Step 3.

Rinsed with PBS (eg. 3 min x 3 times).

Step 4.

Block with 5% (w/v) skim milk in PBS at room temperature for 30 min. (It is also possible to use BSA etc for blocking). Sodium azide (NaN_3, 0.02%w/v) can be included in this skim milke blocker, but it is important to rinse cells extensively at Step 5.

Step 5.

After the blocking solution was removed, incubated with P-RANbody diluted in DMEM10 (DulbeccoMEM plus 10% fetal calf serum, pH7.4) overnight at 4 °C. Do not include sodium azide in this reaction because azide inactivates HRP. If a preservative is required, use ProClin 150 (Millipore-Sigma).

How to use ProClin 150.

http://dshb.biology.uiowa.edu/Antibody-Collections/FAQ#proclin150

https://www.sigmaaldrich.com/catalog/substance/proclin1501234598765?lang=en&region=US

To generate and optimize P-RANbodies, please look at 'Generation and preparation of RANbodies' (in preparation).

Step 6.

Rinse with PBS.

Step 7.

Stain with tyramide dye (TSA Plus kit from Perkin-Elmer). Typically, incubate for 5 min to 30 min. However, it is possible to extend incubation up to 3-4 hours. Do not dry out.

For beginners, these evaluation kits from PerkinElmer are affordable.

TSA Plus Cyanine 3 Evaluation Kit

TSA Plus Fluorescein Evaluation Kit

http://www.perkinelmer.com/category/tissue-biomarker-reagents/tsa-plus/

Step 8.

Rinse stained cells with PBS overnight (just incubate without changing PBS). For the best result, it is important to remove background extensively. If the signal is intense, it might not be required to rinse for a long time.

If the second immunostaining is necessary, start immunostaining from this step. In this case, this long rinsing step can be omitted.

Step 9.

Mount in Fluoro-Gel (Electron Microscopy Sciences), or the similar mounting reagent.

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Warnings

It is important to use hydrogen peroxide (H_2O_2) carefully.

http://www.inchem.org/documents/icsc/icsc/eics0164.htm