Western Blot Protocol for Detection of PPARα, PPARδ or PPARγ2 using PAbs from ALEXIS CORPORATION:

ALX-210-118
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1. Total or nuclear/cytoplasmic protein can be isolated as described in [1]. Cleaner results are obtained with cell extracts than with tissues.
2. A minimum of 50 to 100 µg of protein separated on a 4-20% gradient gel or any gel between 8% and 12% acrylamide (37:1 acrylamide/bisacrylamide), and transferred on PVDF membrane will give better results.
3. Imunoblotting:
   Perform all steps at room temperature with gentle shacking.
   a) Block 1 hour in blocking buffer.
   b) Primary antibody diluted inTTBS/0.1% milk; 0.27 ml/cm^2 of membrane; incubate for 60 minutes
   c) Wash 1 x 5 minutes in TBS; 3 x 6 minutes in TTBS; Wash 1 x 5 minutes in TBS.
   d) Secondary antibody diluted in in TTBS/0.1% milk; incubate for 60 minutes.
   e) Wash 1 x 5 minutes in TBS; 3 x 6 minutes in TTBS; Wash 1 x 5 minutes in TBS.
4. Detection:
   Incubate 1 minute with chemiluminescent reagents (Amersham or NEN) and expose to film. A weak signal should be seen within 1 to 3 minutes. Exposures ranging from 5 to 30 minutes are often necessary to get the desirable signal intensity. In case the signal is to weak, reincubate with secondary antibody and omit one of the 5minutes washes in TTBS. Vice versa if the signal is too strong or if there is high background staining, wash once more in TTBS during the final washes.

Buffers:

TBS 10x (1 liter):
30 g TRIS-base
2 g KCl
80 g NaCl
Adjust pH to 7.4 with HCl.

TTBS:
TBS with 0.25% Tween20

Blocking Buffer:
5% non-fat dry milk in TBS.

[1] *Cytosolic and nuclear distribution of PPARgamma2 in differentiating 3T3-L1 preadipocytes:* P. Thuillier, et al.; J. Lipid Res. 39, 2329 (1998)

The procedures listed above are intended only as a guide. Various assay conditions require that the investigator determine the optimal working concentrations. The results may vary depending on experimental conditions and technique. No warranty or guarantee of performance of above procedure is made or implied. Use good laboratory practices and handle all materials with care.

These products and procedures are for in vitro experimental use only and are not intended for use in humans or clinical diagnosis.