



Fig. 19. B-Raf immunofluorescence in motoneurons from E12.5 *B-raf*^{+/+} and *B-raf*^{-/-} mouse embryos after 24 h culture with BDNF and CNTF. The motoneurons were immunostained with specific antibodies against B-Raf and neurofilament. Some individual motoneurons stained against B-Raf/Cy3 (red) and neurofilament/FITC (green) from *B-raf*^{+/+} mice were selected and analyzed under the confocal laser scanning microscope (50 X, TCS, Leica, Heidelberg, Germany) using single or double filters for visualization of single (red or green) or combined fluorescence (orange). Note that *B-raf*^{+/+} mouse motoneurons show marked expression of B-Raf immunoreactivity (A,B), which is mainly localized in the perinuclear space and the nucleus. The result is similar to the results obtained from wild-type E13 and E14 mice (Fig. 14). In addition, immunodouble-labelling with B-Raf (red, C) and neurofilament (green, D) in *B-raf*^{+/+} mouse motoneurons showed that these two immunoreactivities are colocalized (E) in the same neurons confirming their identity as motoneurons. On the contrary, motoneurons from *B-raf*^{-/-} mice (F) did not survive after 6 h culture.